FORM PTO 1390
(REV 5-93) US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371

ATTORNEY DOCKET NUMBER
2001_0710A

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)
NEW 09/857887

International Application No.	International Filing Date	Priority Date Claimed
PCT/JP00/00023	January 6, 2000	January 7, 1999

Title of Invention
POLYOL COMPOUNDS, THEIR PRODUCTION AND USE

Applicant(s) For DO/EO/US
Keiji KAMIYAMA, Yuji NISHIKIMI, Atsushi HASUOKA, Masafumi NAKAO, Ken-ichiro MIYAGAWA and Yohko AKIYAMA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.

2. [] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.

3. [] This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).

4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau). **ATTACHMENT A**
   b. [X] has been transmitted by the International Bureau.
   c. [] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [] A translation of the International Application into English (35 U.S.C. §371(c)(2)).

7. [] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3)).
   a. [] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] have been transmitted by the International Bureau.
   c. [] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [] have not been made and will not be made.

8. [] A translation of the amendments to the claims under PCT Article 19.

9. [X] An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)). **ATTACHMENT B**

10. [] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 14. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and **1.98. ATTACHMENT C**

12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
    ATTACHMENT D

13. [X] A FIRST preliminary amendment. **ATTACHMENT E**
    [] A SECOND or SUBSEQUENT preliminary amendment.

14. [] Other items or information:

THE COMMISSIONER IS AUTHORIZED TO CHARGE ANY DEFICIENCY IN THE FEE FOR THIS PAPER TO DEPOSIT ACCOUNT NO. 23-0975.

531 Rec'd PCT 13 JUN 2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/857887 NEW	PCT/JP00/00023	2001_0710A

15. [X] The following fees are submitted	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee nor international search fee paid to USPTO and International Search Report not prepared by the EPO or JPO $1000.00		
International Search Report has been prepared by the EPO or JPO ..... $ 860.00		
International preliminary examination fee not paid ot USPTO but international search paid to USPTO ..... $ 710.00		
International preliminary examination fee paid to USPTO but claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00		
International preliminary examination fee paid ot USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	23 -20 =	3	X $18.00	$54.00	
Independent Claims	- 3 =		X $80.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$914.00	
[] Small Entity Status is hereby asserted. Above fees are reduced by 1/2.				$	
SUBTOTAL =				$914.00	
Processing fee of $130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$914.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40 per property +				$40.00	
TOTAL FEES ENCLOSED =				$954.00	
				Amount to be refunded	$
				Amount to be charged	$

a. [X] A check in the amount of $954.00 to cover the above fees is enclosed. A duplicate copy of this form is enclosed.

b. [] Please charge my Deposit Account No. 23-0975 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-0975.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

19. CORRESPONDENCE ADDRESS

000513

PATENT TRADEMARK OFFICE

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Warren M. Cheek, Jr.
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June 13, 2001

[CHECK NO. 44923]

[2001_0710A]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Keiji KAMIYAMA et al. : **Attn: BOX PCT**

Serial No. NEW : **Docket No. 2001_0710A**

Filed June 13, 2001 :

POLYOL COMPOUNDS, THEIR PRODUCTION AND USE
[Corresponding to PCT/JP00/00023
Filed January 6, 2000]

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents,
Washington, DC 20231

Sir:

Prior to calculating the filing fee, please amend the above-identified application as follows:

IN THE SPECIFICATION

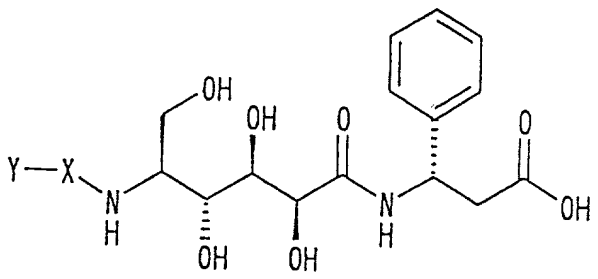
Page 1, immediately after the title, please insert:

This application is a 371 of PCT/JP00/00023 filed January 6, 2000.

IN THE CLAIMS

Please amend the claims as follows:

1. (Amended) A compound of the formula:



(I)

ATTACHMENT E

wherein X is L-serine residue, L-asparagine residue or (S)-2-aminobutyric acid residue and Y is α-L-amino acid residue, or a salt thereof.

7. (Amended) A pharmaceutical composition which comprises the compound claimed in claim 1 or its pro-drug and a pharmaceutically acceptable additive.

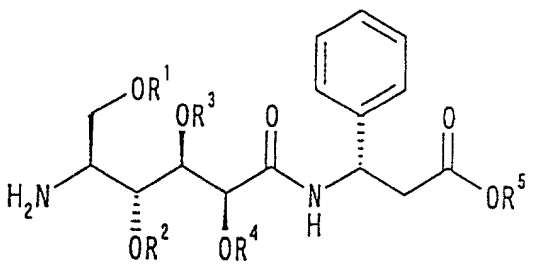
12. (Amended) A pharmaceutical composition which is a gastric mucosa adhesive pharmaceutical composition comprising (a) a compound as claimed in claim 1, (b) a lipid and/or a polyglycerol fatty acid ester and (c) a viscogenic agent capable of being viscous with water.

19. (Amended) A method for manufacturing a pharmaceutical composition for *Helicobacter pylori* infectious disease, which comprises mixing the compound according to claim 1 or its pro-drug with a pharmaceutically acceptable additive.

20. (Amended) The method as claimed in claim 19, wherein the composition is for treating or preventing a *Helicobacter pylori* infectious disease.

21. (Amended) The method as claimed in claim 20, wherein the *Helicobacter pylori* infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

22. (Amended) A method for producing a compound claimed in claim 1, which comprises reacting a compound of the formula:



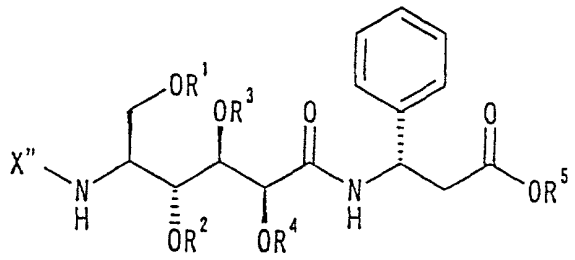
(II)

wherein R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom, a salt thereof or a reactive derivative thereof at the amino group, with a compound of the formula:

$$Y'—X'— OH \qquad (III)$$

wherein X' is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, and Y' is α-L-amino acid residue which may be protected, a salt thereof or a reactive derivative thereof at the carboxyl group, if necessary, followed by removing the protecting group.

23. (Amended) A method for producing a compound claimed in claim 1, which comprises reacting a compound of the formula:



(IV)

wherein X" is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom, a salt thereof or a reactive derivative thereof at the amino group, with a compound of the formula:

$$Y'— OH \qquad (V)$$

wherein Y' is α-L-amino acid residue which may be protected, a salt thereof or a reactive derivative thereof at the carboxyl group, if necessary, followed by removing the protecting group.

REMARKS

The specification has been amended to reflect the 371 status.

The claims have been amended to better conform to U.S. practice. Specifically, claims 1, 22 and 23 have been amended to remove the brackets. Claim 7 has been amended to recite a second component of the pharmaceutical composition, a pharmaceutically acceptable additive. Support is found in the specification at page 29, lines 8-12. Claim 12 has been amended to remove the multiple dependency to reduce the PTO filing fee. Claims 19-21 have been rewritten in U.S. format.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version with markings to show changes made**".

Favorable action on the merits is solicited.

Respectfully submitted,

Keiji KAMIYAMA et al.

By _____
Warren M. Cheek, Jr.
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Attorney for Applicants

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June 13, 2001

DESCRIPTION

POLYOL COMPOUNDS, THEIR PRODUCTION AND USE

This application is a 371 of PCT/JP00/00023 filed January 6, 2000.

TECHNICAL FIELD

The present invention relates to a polyol, a method of its production, and its use. More particularly, the invention relates to a bioactive compound of use as a medicine, for as a preventing and treating drug for diseases such as gastric ulcer and duodenal ulcer, and an anti-Helicobacter pylori agent containing the said compound.

BACKGROUND ART

Being a member of the group of bacteria doing harm in the gastrointestinal tract, Helicobacter pylori is a gram-negative microaerophile belonging to the genus Helicobacter and, as suggested, may be a major factor in the recurrences of gastritis, duodenal ulcer and stomach ulcer.

For the treatment of various diseases associated with Helicobacter pylori infection, chemotherapy such as a two-drug combined therapy using a bismuth drug and an antibiotic or a three-drug combined therapy using a bismuth drug, metronidazole (US Patent 2,944,061), and either tetracycline (e.g. US Patent 2,712,517) or amoxicillin (US Patent 3,192,198) is being practiced today. The ternary therapy consisting of a gastric proton pump inhibitor, amoxicillin, and clarithromycin has also been found to be effective (Gut, 1995, 37 (Supplement 1) : A365) (Gastroenterology, 1996, 110 : A171). Such drugs as bismuth drugs, antibiotics, and metronidazole are all administered by the oral route.

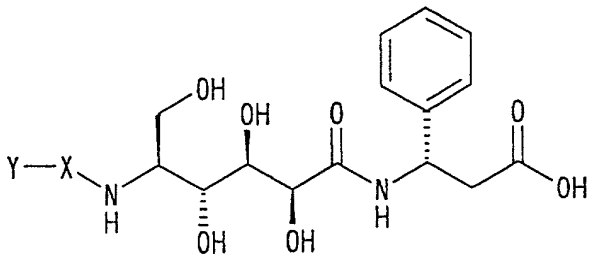
Referring to polyols, PCT International Patent Application Publication No. WO93/06838 and Acta Chemical Scandinavica B 36, 515-518 (1982) disclose

Version with Markings to Show Changes Made

CLAIMS

(Amended)

1. A compound of the formula:



(I)

wherein X is L-serine residue, L-asparagine residue or (S)-2-aminobutyric acid residue and Y is α-L-amino acid residue, or ~~its~~ a salt thereof.

2. A compound as claimed in claim 1, wherein X is (S)-2-aminobutyric acid residue.

3. A compound as claimed in claim 1, wherein Y is norvaline residue, isoleucine residue or methionine residue.

4. A compound as claimed in claim 1, which is (S)-3-[(2S,3R,4R,5S)-5-(L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid or its salt.

5. A compound as claimed in claim 1, which is (S)-3-[(2S,3R,4R,5S)-5-(L-isoleucyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid or its salt.

6. A pro-drug of the compound claimed in claim 1.

(Amended)

7. A pharmaceutical composition which contains the compound claimed in claim 1 or its pro-drug and a pharmaceutically acceptable additive.

8. A pharmaceutical composition as claimed in claim 7, which is an anti-Helicobacter pylori agent.

9. A pharmaceutical composition as claimed in claim 8, which is a preventing and treating agent of Helicobacter pylori infectious disease.

10. A pharmaceutical composition as claimed in claim 9, wherein Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

11. A pharmaceutical composition as claimed in claim 7, which

is a gastric mucosa adhesive pharmaceutical composition.

12. (Amended) A pharmaceutical composition ~~as claimed in claim 11, wherein~~ which is a gastric mucosa adhesive pharmaceutical composition ~~contains~~ comprising (a) a compound as claimed in claim 1, (b) a lipid and/or a polyglycerol fatty acid ester and (c) a viscogenic agent capable of being viscous with water.

13. A pharmaceutical composition as claimed in claim 12, wherein (c) the viscogenic agent is an acrylic polymer.

14. A pharmaceutical composition as claimed in claim 12, which further contains (d) a material which swells the viscogenic agent.

15. A pharmaceutical composition as claimed in claim 14, (d) the material which swells the viscogenic agent is curdlan and/or a low-substituted hydroxypropylcellulose.

16. A pharmaceutical composition which contains both of a compound as claimed in claim 1 or its pro-drug and the other antibacterial agent and/or an antiulcerative agent.

17. A method for treating or preventing a mammal suffering from a Helicobacter pylori infectious disease, which comprises administering an effective amount of a compound according to claim 1 or its pro-drug optionally together with a pharmaceutically acceptable carrier, diluent or excipient, to a patient suffering from the disease.

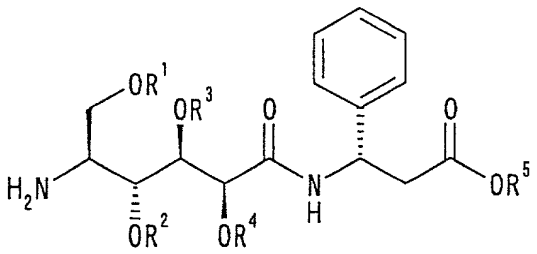
18. A method as claimed in claim 17, wherein Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

19. (Amended) ~~Use of the compound according to claim 1 or its pro-drug for~~ A method for manufacturing ~~of~~ a pharmaceutical composition for a Helicobacter pylori infectious disease, which comprises mixing the compound according to claim 1 or its pro-drug with a pharmaceutically acceptable additive.

20. (Amended) ~~Use~~ the method as claimed in claim 19, wherein the composition is for treating or preventing a Helicobacter pylori infectious disease.

21. (Amended) ~~Use~~ the method as claimed in claim 20, wherein the Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

22. (Amended) A method for producing a compound claimed in claim 1, which comprises reacting a compound of the formula:



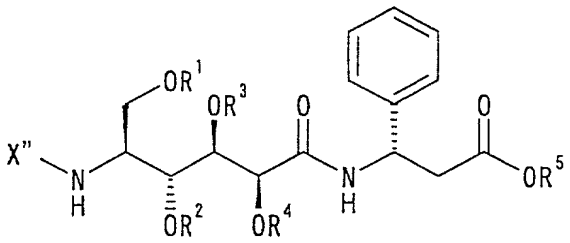
(II)

wherein R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom, ~~its~~ a salt thereof or ~~its~~ a reactive derivative thereof at the amino group, with a compound of the formula:

$$Y'—X'—OH \qquad (III)$$

wherein X' is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, and Y' is α-L-amino acid residue which may be protected, ~~its~~ a salt thereof or ~~its~~ a reactive derivative thereof at the carboxyl group, if necessary, followed by removing the protecting group.

(Amended) 23. A method for producing a compound claimed in claim 1, which comprises reacting a compound of the formula:



(IV)

wherein X" is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom, ~~its~~ a salt thereof or ~~its~~ a reactive derivative thereof at the amino group, with a compound of the formula:

$$Y'—OH \qquad (V)$$

wherein Y' is α-L-amino acid residue which may be protected, ~~its~~ a salt thereof or ~~its~~ a reactive derivative thereof at the carboxyl group, if necessary, followed by removing the protecting group.

DESCRIPTION

POLYOL COMPOUNDS, THEIR PRODUCTION AND USE

TECHNICAL FIELD

The present invention relates to a polyol, a method of its production, and its use. More particularly, the invention relates to a bioactive compound of use as a medicine, for as a preventing and treating drug for diseases such as gastric ulcer and duodenal ulcer, and an anti-Helicobacter pylori agent containing the said compound.

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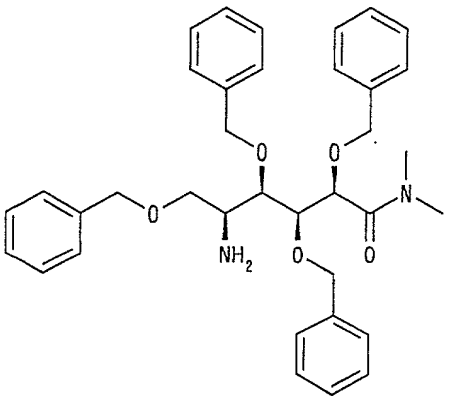
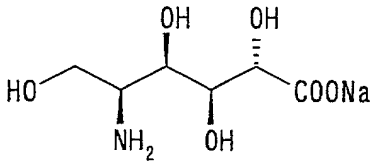
For the treatment of various diseases associated with Helicobacter pylori infection, chemotherapy such as a two-drug combined therapy using a bismuth drug and an antibiotic or a three-drug combined therapy using a bismuth drug, metronidazole (US Patent 2,944,061), and either tetracycline (e.g. US Patent 2,712,517) or amoxicillin (US Patent 3,192,198) is being practiced today. The ternary therapy consisting of a gastric proton pump inhibitor, amoxicillin, and clarithromycin has also been found to be effective (Gut, 1995, 37 (Supplement 1) : A365) (Gastroenterology, 1996, 110 : A171). Such drugs as bismuth drugs, antibiotics, and metronidazole are all administered by the oral route.

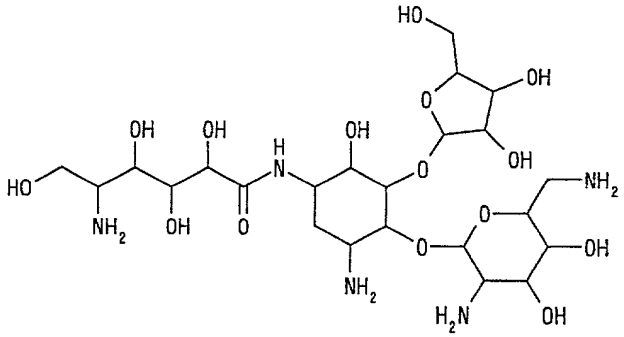
Referring to polyols, PCT International Patent Application Publication No. WO93/06838 and Acta Chemical Scandinavica B 36, 515-518 (1982) disclose



and



respectively, as synthetic intermediates, and Carbohyd. Res., 28 (2), 263-280 (1973) states that



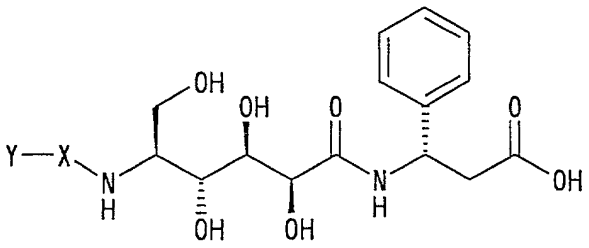
is active against gram-negative bacteria.

For an improved expression of the efficacy of an active ingredient and a reduced risk for side effects, an attempt was made to formulate amoxicillin, for instance, into a gastric mucosa-adhesive composition to prolong its intragastric residence time and let amoxicillin be released at a controlled rate and with consequent improved availability of active ingredients (WO 94/00112). It has been demonstrated that the rate of clearance of Helicobacter pylori can be improved by causing an anti-Helicobacter pylori substance to stay in the stomach longer to ensure prolonged exposure of the bacteria to the active substance [Scand. J. Gastroenterol., 29, 16-42 (1994)].

However, in order that a sufficient growth-inhibitory concentration may be maintained in the habitat of Helicobacter pylori, said bismuth drugs, antibiotics, or metronidazole must be administered daily in massive doses and such therapeutics entail various troubles, for example, the onset of adverse reactions such as vomiting and diarrhea. Under the circumstances, the present invention has for its object to provide a novel medicinal agent having high antibacterial activity, particularly against Helicobacter pylori and other bacteria of the genus Helicobacter, and producing clinically rewarding preventing and treating responses with a reduced incidence of adverse reactions.

DISCLOSURE OF INVENTION

As the result of their intensive research, the inventors of the present invention synthesized a novel polyhydric alcohol (polyol) of the following formula :



(I)

wherein X is L-serine residue, L-asparagine residue or (S)-2-aminobutyric acid residue and Y is α-L-amino acid residue, and discovered that, because of this unique chemical structure that dipeptide Y-X is bonded directly to nitrogen atom, the above compound displays remarkable inhibitory activity against the bacteria doing harm in the gastrointestinal tract, particularly high anti-Helicobacter activity, with clinically favorable pharmacological characteristics such as a low risk for adverse effects. The present invention has been developed on the basis of the above finding.

In view of the above state of the art, the inventors of the present invention have discovered that the effectiveness of active ingredients (e.g. anti Helicobacter pylori effect) can be potentiated by administering gastric mucosa adhesive composition

containing an active ingredient (e.g. anti Helicobacter pylori substance), and that the composition has favorable safety characteristics and an enhanced adhesion to the mucosa.

Thus the present invention relates to :

(1) A compound of the formula (I) or its salt;

(2) A compound as shown in the above (1), wherein X is (S)-2-aminobutyric acid residue;

(3) A compound as shown in the above (1), wherein Y is norvaline residue, isoleucine residue or methionine residue;

(4) A compound as shown in the above (1), which is (S)-3-[(2S,3R,4R,5S)-5-(L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid or its salt;

(5) A compound as shown in the above (1), which is (S)-3-[(2S,3R,4R,5S)-5-(L-isoleucyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid or its salt;

(6) A pro-drug of the compound or its salt shown in the above (1);

(7) A pharmaceutical composition which contains a compound as shown in the above (1) or its pro-drug;

(8) A pharmaceutical composition as shown in the above (7), which is an anti-Helicobacter pylori agent;

(9) A pharmaceutical composition as shown in the above (8), which is a preventing and treating agent of Helicobacter pylori infectious disease;

(10) A pharmaceutical composition as shown in the above (9), wherein Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma;

(11) A pharmaceutical composition as shown in the above (7), which is a gastric mucosa adhesive pharmaceutical composition;

(12) A pharmaceutical composition as shown in the above (11), wherein a gastric mucosa adhesive pharmaceutical composition contains (a) a compound as shown in the above (1), (b) a lipid and/or a polyglycerol fatty acid ester and (c) a viscogenic agent capable of being viscous with water;

(13) A pharmaceutical composition as shown in the above (12), wherein (c) the viscogenic agent is an acrylic polymer;

(14) A pharmaceutical composition as shown in the above (12), which further contains (d) a material which swells the viscogenic agent;

(15) A pharmaceutical composition as shown in the above (14), (d) the material which swells the viscogenic agent is curdlan and/or a low-substituted hydroxypropylcellulose;

(16) A pharmaceutical composition which contains both of a compound as shown in the above (1) and the other antibacterial agent and/or an antiulcerative agent;

(17) A method for treating or preventing a mammal suffering from a Helicobacter pylori infectious disease, which comprises administering an effective amount of a compound as shown in the above (1) or its pro-drug optionally together with a pharmaceutically acceptable carrier, diluent or excipient, to a patient suffering from the disease;

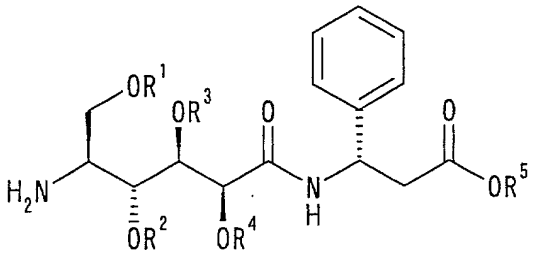
(18) A method as shown in the above (17), wherein Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma;

(19) Use of the compound as shown in the above (1) or its pro-drug for manufacturing of a pharmaceutical composition for a Helicobacter pylori infectious disease;

(20) Use as shown in the above (19), wherein the composition is for treating or preventing a Helicobacter pylori infectious disease;

(21) Use as shown in the above (20), wherein the Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma;

(22) A method for producing a compound as shown in the above (1), which comprises reacting a compound of the formula :



(II)

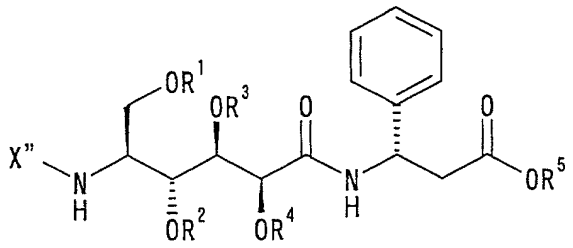
[wherein R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom], its salt or its reactive derivative at the amino group with a compound of the formula :

$$Y'—X'—OH \qquad (III)$$

[wherein X' is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, Y' is α-L-amino acid residue which may be protected], its salt or its reactive derivative at the carboxyl group, if necessary, followed by removing the protecting group;

and

(23) A method for producing a compound as shown in the above (1), which comprises reacting a compound of the formula :



(IV)

[wherein X" is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, and the other symbols have the meanings given above], its salt or its reactive derivative at the amino group with a compound of the formula :

$$Y'—OH \qquad (V)$$

[wherein Y' has the meaning given above], its salt or its reactive derivative at the carboxyl group, if necessary, followed by removing the protecting group.

In the above formula (I), L-serine residue, L-asparagine residue and (S)-2-aminobutyric acid residue, each of which is

represented by X, respectively mean parts which form by removing a hydroxy group from the carboxyl group and a hydrogen atom from the amino group of L-serine, L-asparagine and (S)-2-aminobutyric acid. In the above formula (I), these amino acid residues represented by X are bonded with the amino group of 5-amino-2,3,4,6-tetrahydroxyhexanoyl group at the carbonyl group which forms by removing a hydroxy group from the carboxyl group, and bond with Y at NH which forms by removing a hydrogen atom from the amino group. The symbol X is preferably (S)-2-aminobutyric acid residue.

In the above formula (I), α-L-amino acid residue represented by Y means a part which forms by removing a hydroxy group from the carboxyl group of α-L-amino acid, and bonds to X at the carbonyl group which forms by removing a hydroxy group from the carboxyl group. The "α-L-amino acid" include such amino acids as alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, etc. and such other amino acids as norvaline, norleucine, 2-aminoadipic acid, 2-aminobutyric acid, 2-aminoisobutyric acid, 2-amino-4-pentenoic acid, 1-aminocyclopropanecarboxylic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclohexanecarboxylic acid, thyronine, ornithine, hydroxyproline, hydroxylysine, (2-naphthyl)alanine, azaglycine, etc. Among them, norvaline, isoleucine and methionine, etc. are preferable.

In the above formulas (II) and (IV), the protecting group for hydroxy group represented by R^1, R^2, R^3 and R^4 means one usually known as a protecting group for hydroxy group in the field of peptide chemistry. The protecting group for the hydroxy includes ether-forming protecting groups such as tert-butyl, methoxymethyl, benzyloxymethyl, tert-butoxymethyl, 2-methoxyethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, methylthiomethyl, 2-tetrahydropyranyl, 4-methoxy-4-tetrahydropyranyl, 2-tetrahydropyranyl, benzyl, p-methoxybenzyl, p-nitrobenzyl, o-nitrobenzyl, 2,6-dichlorobenzyl, trityl, isopropylidene, cyclohexylidene, benzylidene, p-methoxybenzylidene, etc.; silyl

ether-forming protecting groups such as trimethylsilyl, triethylsilyl, triisopropylsilyl, isopropyldimethylsilyl, diethylisopropylsilyl, tert-butyldimethylsilyl, tert-butyldiphenylsilyl, tribenzylsilyl, triphenylsilyl, methyldiphenylsilyl, di-tert-butylsilylene, etc.; and ester-forming protecting groups such as formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, pivaloyl, benzoyl, benzyloxycarbonyl, 2-bromobenzyloxycarbonyl, cyclic carbonate, etc. Among them, acetyl is preferable.

In the above formulas (II) and (IV), the protecting group for carboxyl group represented by R^5 means one usually known as a protecting group for carboxyl group in the field of peptide chemistry.

The carboxyl-protecting group which can be used includes ester-forming protecting groups such as methyl, ethyl, methoxymethyl, methoxyethoxymethyl, benzyloxymethyl, tert-butyl, benzyl, p-methoxybenzyl, p-nitrobenzyl, o-nitrobenzyl, benzhydryl, trityl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, allyl, cyclohexyl, cyclopentyl, phenacyl, etc.; silyl ester-forming protecting groups such as trimethylsilyl, triethylsilyl, tert-butyldimethylsilyl, isopropyldimethylsilyl, dimethylphenylsilyl, etc. Among them, benzhydryl, etc. are preferable.

In the above formula (III), L-serine residue which may be protected, L-asparagine residue which may be protected and (S)-2-aminobutyric acid residue, each of which is represented by X', respectively mean parts which form by removing a hydroxy group from the carboxyl group and a hydrogen atom from the amino group of L-serine residue which may be protected, L-asparagine residue which may be protected and (S)-2-aminobutyric acid residue. In the above formula (III), L-serine residue which may be protected, L-asparagine residue which may be protected and (S)-2-aminobutyric acid residue represented by X' respectively includes, L-serine residue which is not protected, L-asparagine residue which is not protected and (S)-2-aminobutyric acid residue, and also include L-serine residue whose hydroxy group is protected, L-asparagine residue whose carbamoyl group is protected. L-serine residue which

is protected is one whose hydroxy group is protected and L-asparagine residue which is protected is one whose carbamoyl group is protected. The protecting group for the hydroxy group of L-serine residue includes those mentioned as the protecting group for the hydroxy group shown by R^1, R^2, R^3 and R^4. Among them, tert-butyl group, etc. is preferable. The protecting group for the carbamoyl group of L-asparagine residue include, for example, xanthyl group, 4-methoxybenzyl group, 2,4-dimethoxybenzyl group, benzhydryl group, 4,4'-dimethoxybenzhydryl group, etc. Among them, triphenylmethyl group is preferable.

In the above formula (IV), L-serine residue which may be protected, L-asparagine residue which may be protected and (S)-2-aminobutyric acid residue, each of which is represented by X", respectively mean group which forms by removing a hydroxy group from the carboxyl group of L-serine which may be protected, L-asparagine which may be protected and (S)-2-aminobutyric acid. In the above formula (IV), X" bond to amino group of 5-amino-2,3,4,6-tetrahydroxyhexanoyl group at carbonyl group which forms by removing a hydroxy group from the carboxyl group. In the above formula (IV), L-serine residue which may be protected, L-asparagine residue which may be protected and (S)-2-aminobutyric acid residue , each of which is represented by X" include L-serine residue which is not protected, L-asparagine residue which is not protected and (S)-2-aminobutyric acid residue and also include L-serine residue whose hydroxy group is protected, L-asparagine residue whose carbamoyl group is protected. The protecting group for the hydroxy group of L-serine residue and the protecting group for the carbamoyl group of L-asparagine residue include those mentioned for X'.

In the above formulas (III) and (V), α-L-amino acid residue which may be protected, represented by Y', includes α-L-amino acid residue which may be protected, represented by Y, and also includes ones whose amino group, carboxyl group, hydroxy group and carbonyl group are partially or entirely protected, when α-L-amino acid residue has a carboxyl group, a hydroxy group or a carbonyl group. The protection for the amino group, carboxyl group, hydroxy group

and carbonyl group, means those by the protecting group usually known as the protecting group for amino group, protecting group for carboxyl group, protecting group for hydroxy group and protecting group for carbonyl group in the field of peptide chemistry.

The amino-protecting group which can be used includes amide-forming protecting groups such as formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, trifluoroacetyl, acetoacetyl, o-nitrophenylacetyl, etc.; carbamate-forming protecting groups such as tert-butoxycarbonyl, benzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, benzhydryloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-trimethylsilylethoxycarbonyl, 1-methyl-1-(4-biphenyl)ethoxycarbonyl, 9-fluorenylmethoxycarbonyl, 9-anthrylmethoxycarbonyl, isonicotinyloxycarbonyl, 1-adamantyloxycarbonyl, etc.; trityl, and phthaloyl. Among them, tert-butoxycarbonyl, benzyloxycarbonyl, 9-fluorenylmethoxycarbonyl, etc. are preferable.

The carbonyl-protecting group includes acetal-, ketal-, dithioacetal- or dithioketal-forming protecting groups, such as dimethyl, diethyl, dibenzyl, diacetyl, etc.; protecting groups forming optionally substituted 1,3-dioxane or 1,3-dioxolane, protecting groups forming 1,3-dithiane or 1,3-dithiolane, and protecting groups forming hydrazones substituted with N,N-dimethyl, 2,4-dinitrophenyl, etc. Among them, 1,3-dioxane, etc. are preferable.

The protecting group for carboxyl group include those mentioned for the carboxyl group represented by R^5. Among them, tert-butyl group, benzyl group, etc. are preferable. The protecting group for the hydroxy group include those mentioned for the hydroxy group represented by the above R^1, R^2, R^3 and R^4.

The salt of Compound (I) according to the invention includes a salt with a pharmacologically acceptable base and a salt with an pharmacologically acceptable acid. The salt with a pharmacologically acceptable base include a salt with an alkali metal (e.g. sodium, potassium, etc.) and a salt with an alkaline

earth metal (e.g. calcium, magnesium, etc.). The pharmacologically acceptable acid include a salt with an inorganic acid (e.g. hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, etc.) and a salt with an organic acid (e.g. acetic acid, propionic acid, lactic acid, succinic acid, maleic acid, tartaric acid, citric acid, gluconic acid, ascorbic acid, benzoic acid, methanesulfonic acid, p-toluenesulfonic acid, cinnamic acid, fumaric acid, malic acid, oxalic acid, etc.).

The pro-drug of the compound of the formula (I) or its salt [herein after referred to as Compound (I)]means a compound which is converted to Compound (I) under the physiological condition or with a reaction due to an enzyme, an gastric acid, etc. in the living body, that is, a compound which is converted to Compound (I) with oxidation, reduction, hydrolysis, etc. according to an enzyme; a compound which is converted to Compound (I) with gastric acid, etc.

Examples of the pro-drug of Compound (I) include a compound wherein an amino group of Compound (I) is acylated, alkylated, phosphorylated, etc. (e.g. a compound wherein an amino group of Compound (I) is eicosanoylated, alanylated, pentylaminocarbonylated, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxycarbonylated, tetrahydrofuranylated, pyrrolidylmethylated, pivaloyloxymethylated, tert-butylated, etc.); a compound wherein an hydroxy group of Compound (I) is acylated, alkylated, phosphorylated, borylated, etc. (e.g. a compound wherein an hydroxy group of Compound (I) is acetylated, palmitoylated, propanoylated, pivaloylated, succinylated, fumarylated, alanylated, dimethylaminomethylcarbonylated, etc.); a compound wherein a carboxyl group of Compound (I) is modified with ester, amide, etc. (e.g. a compound wherein a carboxyl group of Compound (I) is modified with ethyl ester, phenyl ester, carboxymethyl ester, dimethylaminomethyl ester, pivaloyloxymethyl ester, ethoxycarbonyloxyethyl ester, phthalidyl ester, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester, cyclohexyloxycarbonylethyl ester, methyl amide, etc.); etc. These pro-drug can be produced by per se known method from Compound (I), (II) and (III).

The pro-drug of Compound (I) may be a compound which is converted into Compound (I) under the physiological conditions as described in "Pharmaceutical Research and Development", Vol. 7 (Drug Design), pages 163-198 published in 1990 by Hirokawa Publishing Co. (Tokyo, Japan).

Also, Compound (I) may be hydrated.

The production of Compound (I) in the present invention is mentioned below.

Compound (I) can be produced, for example, by reacting a compound of the formula (II), its salt or its reactive derivative at the amino group [hereinafter briefly referred to as Compound (II)] with a compound of the formula (III), its salt or its reactive derivative at the carboxyl group [hereinafter briefly referred to as Compound (III)], or by reacting a compound of the formula (IV), its salt or its reactive derivative at the amino group [hereinafter briefly referred to as Compound (IV)] with a compound of the formula (V), its salt or its reactive derivative at the carboxyl group [hereinafter briefly referred to as Compound (V)], if necessary, followed by deprotection.

In the above Compounds (II) and (IV), the reacting derivatives at the amino group mean ones capable of forming peptide bond by reacting with compounds (III) or (V) respectively, for example, a compound which forms by the substitution of the amino group of Compound (II) and Compound (IV) etc. with a trimethylsilyl group, a trimethylstannyl group.

In the above Compounds (III) and (V), the reacting derivatives at the carboxyl group mean ones capable of forming peptide bond by reacting with compounds (II) and (IV) respectively, and can be prepared from Compound (III) and (V) respectively, for example, by the acid halide method, azide method, mixed acid anhydride method (the "counterpart acid" which can be used includes isobutyloxycarbonyl chloride, pivaloyl chloride, etc.), symmetric acid anhydride method, the method using a condensing agent such as N,N'-carbodiimidazole, N,N'-dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-ethoxycarbonyl-2-ethoxy-

1,2-dihydroquinoline, diethyl phosphorocyanidate, diphenylphosphoryl azide, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate, bromotrispyrrolidinophosphonium hexafluorophosphate, 2-(5-norbornene-2,3-dicarboximido)tetramethyluronium tetrafluoroborate, or the like, the method which comprises using the above condensing agent in the presence of 4-dimethylaminopyridine, 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazole, N-hydroxysuccinimide, N-hydroxy-5-norbornene-2,3-dicarboximide, 1-hydroxybenzotriazole or the like, or the active ester method using them.

The reaction between Compound (II) with Compound (III) and the reaction between Compound (IV) with Compound (V) can usually be carried out in the presence of a solvent.

The solvent which can be used includes aromatic hydrocarbons such as benzene, toluene, xylene, etc.; halogenated hydrocarbons such as dichloromethane, chloroform, etc., saturated hydrocarbons such as hexane, heptane, cyclohexane, etc.; ethers such as diethyl ether, tetrahydrofuran, dioxane, etc.; nitriles such as acetonitrile etc.; sulfoxides such as dimethyl sulfoxide etc.; amides such as N,N-dimethylformamide etc.; esters such as ethyl acetate etc., and water. Those solvents can be used each alone or in a combination of 2 or more species, for example in a ratio of 1 : 1 through 1 : 10. Each amount of Compounds (III) and (IV) is 0.5 to 10 equivalents relative to one equivalent of Compounds (IV) and (V), respectively. The reaction temperature is usually about-80 to 100°C and preferably about-50 to 50°C. The reaction time may range from about 1 to 96 hours, preferably about 1 to 72 hours.

In the above reaction, when amino group, carboxyl group, hydroxy group or carbonyl group each of which is not concerned with the reaction is protected, the compound can be subjected to deprotection reaction to convert to Compound (I).

The technology for removing such amino-protecting,

hydroxy-protecting, carbonyl-protecting, and carboxy-protecting groups includes the method using an acid, the method using a base, the reduction method, the ultraviolet method, the hydrazine method, the phenylhydrazine method, the sodium N-methyldithiocarbamate method, the tetrabutylammonium fluoride method, the palladium acetate method, the mercury chloride method, and the Lewis acid method. Those routine methods and/or other known methods can be selectively used.

The method using an acid is one of the common methods for hydrolyzing an amide, ester, silyl ester, or silyl ether, and is applied to elimination of the corresponding type of protecting group. For example, the method is commonly used for deprotection of an amino group protected by tert-butoxycarbonyl, p-methoxybenzyloxycarbonyl, benzhydryloxycarbonyl, 9-anthrylmethoxycarbonyl, 1-methyl-1-(4-biphenyl)ethoxycarbonyl, adamantyloxycarbonyl, or trityl and the deprotection of a hydroxy group protected by methoxymethyl, tert-butoxymethyl, 2-tetrahydropyranyl, 4-methoxy-4-tetrahydropyranyl, 2-tetrahydrofuranyl, or trityl. The preferred acid includes organic acids such as formic acid, trifluoroacetic acid, benzenesulfonic acid, p-toluenesulfonic acid, etc. and inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, etc.

The method using a base, like the above method using an acid, is one of the common methods for hydrolyzing an amide, ester, or the like bond and is applied to elimination of the corresponding type of protecting group. For example, organic bases can be used with advantage for deprotection of an amino group protected by 9-fluorenylmethoxycarbonyl. The preferred base includes such inorganic bases as alkali metal hydroxides, e.g. lithium hydroxide, sodium hydroxide, potassium hydroxide, etc.; alkaline earth metal hydroxides, e.g. magnesium hydroxide, calcium hydroxide, etc.; alkali metal carbonates, e.g. sodium carbonate, potassium carbonate, etc.; alkaline earth metal carbonates, e.g. magnesium carbonate, calcium carbonate, etc.; alkali metal hydrogencarbonates, e.g. sodium hydrogencarbonate, potassium hydrogencarbonate, etc.; alkali metal acetates, e.g. sodium acetate, potassium acetate,

etc.; alkaline earth metal phosphates, e.g. calcium phosphate, magnesium phosphate, etc.; alkali metal hydrogenphosphate, e.g. disodium hydrogenphosphate, dipotassium hydrogenphosphate, etc.; and aqueous ammonia; and such organic bases as trimethylamine, triethylamine, diisopropylethylamine, pyridine, picoline, N-methylpyrrolidine, piperidine, N-methylpiperidine, N-methylmorpholine, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]-7-undecene, etc.

The reduction method is used typically for the deprotection of an amino group protected by trichloroacetyl, trifluoroacetyl, o-nitrophenylacetyl, 2,2,2-trichloroethoxycarbonyl, benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, isonicotinyloxycarbonyl, trityl, or the like; the deprotection of a hydroxy group protected by benzyl, p-nitrobenzyl, or the like; and the protection of a carboxyl group protected by benzyloxymethyl, benzyl, p-nitrobenzyl, phenacyl, 2,2,2-trichloroethyl, benzhydryl, or the like. The preferred mode of reduction includes reduction with sodium borohydride, reduction with zinc/acetic acid, and catalytic reduction.

The ultraviolet method is applied typically to the deprotection of a hydroxy or carboxyl group protected by o-nitrobenzyl.

The hydrazine method is typically applied to the deprotection of an amino group protected by phthaloyl (e.g. phthalimide group).

The phenylhydrazine method is typically applied to the deprotection of an amino group protected by acetoacetyl.

The sodium N-methyldithiocarbamate method is typically applied to the deprotection of a chloroacetyl-protected amino or hydroxy group.

The tetrabutylammonium fluoride method is typically used for deprotecting a 2-trimethylsilylethylcarbamate, silyl ether, or silyl ester to regenerate an amino group, a hydroxy group or a carboxyl group as the case may be.

The palladium acetate method is typically used for deprotecting an allyl ester to regenerate a carboxyl group.

The mercury chloride method is typically applied to the

deprotection of a hydroxy group protected by methylthiomethyl.

The Lewis acid method is typically applied to the deprotection of a hydroxy group protected by 2-methoxyethoxymethyl. The preferred Lewis acid includes zinc bromide and titanium tetrachloride, among other compounds.

The intermediates, reaction products, and end products as produced by the above series of reactions can be isolated and purified as necessary by known purification procedures or procedures analogous thereto, for example by concentration, concentration under reduced pressure, solvent extraction, crystallization, recrystallization, redistribution, and chromatography.

(S)-3-[(2S,3R,4R,5S)-5-Amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid [compound (II) wherein $R^1=R^2=R^3=R^4=R^5=H$] can be produced by reacting (S)-3-[(2S,3R,4R,5S)-5-(L-leucyl)amino-2,3,4,6-tetrahydroxy-hexanoyl]amino-3-phenylpropionic acid (HC-70III), which is a natural substance obtained, for example, by enzyme method, with Actinase E. Compound (II) wherein R^1 to R^4 is a protecting group for a hydroxy group and Compound (II) wherein R^5 is a protecting group for the carboxyl group can be produced from (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid [compound (II) wherein $R^1=R^2=R^3=R^4=R^5=H$] by introducing a protecting group known in the field of peptide chemistry. For example, a compound (II) wherein $R^5=CHPh_2$, $R^1=R^2=R^3=R^4=H$ can be produced by reacting (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid with diphenyldiazomethane to convert the carboxyl group to benzhydryloxycarbonyl group.

Compound (II) wherein $R^5=CHPh_2$, $R^1=R^2=R^3=R^4=COCH_3$ can be produced, for example, by protecting the amino group of Compound (II) wherein $R^5=CHPh_2$, $R^1=R^2=R^3=R^4=H$ with 9-fluorenylmethoxycarbonyl group, and by acetylating four hydroxy groups with acetic anhydride, followed by removing the protecting group for amino group.

The above Compound (III) can be produced by reacting α-L-amino acid whose functional group such as amino group, hydroxy group, carbonyl group, etc. other than carboxyl group which participate the reaction with L-serine whose carboxyl group and hydroxy group may be protected, with L-asparagine whose carboxyl group and carbamoyl group may be protected or with (S)-2-aminobutyric acid whose carboxyl group may be protected, then by removing the protecting group for carboxyl group, if necessary, followed by removing the protecting group for the other functional groups.

The above Compound (IV) can be produced, for example, by reacting Compound (II) obtained by the method shown above with L-serine whose amino group is protected and whose hydroxy group may be protected, with L-asparagine whose amino group is protected and whose carbamoyl group may be protected or with (S)-2-aminobutyric acid whose amino group is protected and then removing the protecting group for amino group.

The above Compound (V) wherein Y' is α-L-amino acid residue which is protected can be produced, for example, by protecting functional groups other than carboxyl group of α-L-amino acid by a conventional method.

Compound (I) according to the present invention is less toxic and has laudable pharmacobiological activities, for example high antibacterial activity against Helicobacter bacteria represented by Helicobacter pylori, so that it is effective in the prevention or treatment of diseases associated with Helicobacter pylori infection and/or an ammonium produced by Helicobacter pylori (e.g., duodenal ulcer, gastric ulcer, gastritis (inclusive of chronic gastritis), cancer of the stomach, gastric MALT lymphoma, hepatic encephalopathy, diabetes mellitus, urticaria), especially duodenal ulcer, gastritis, gastric MALT lymphoma.

Also, in the preparation of the present invention, compound (I) or its salt can be used in combination with other antibacterial agents and antiulcer agents.

Other antibacterial agents that can be used in combination with compound (I) include, for example, nitroimidazole antibiotics (e.g.,

tinidazole and metronidazole), tetracyclines (e.g., tetracycline, doxycycline and minocycline), penicillins (e.g., amoxicillin, ampicillin and mezlocillin), cephalosporins (e.g., cefaclor, cefadroxil, cefazolin, cefuroxime, cefuroxime axetil, cephalexin, cefpodoxime proxetil, ceftazidime and ceftriaxone), carbapenems (e.g., imipenem and meropenem), aminoglycosides (e.g., paromomycin), macrolide antibiotics (e.g., erythromycin, clarithromycin and azithromycin), lincosamide antibiotics (e.g., clindamycin), rifamycins (e.g., rifampicin) and nitrofurantoin. Antiulcer agents that can be used in combination with compound (I) include, for example, proton pump inhibitors (e.g., lansoprazole, omeprazole, pantoprazole, rabeprazole, leminoprazole, etc.) and Histamine H_2 antagonists (e.g., ranitidine, cimetidine and famotidine).

The above-described other antibacterial agents and antiulcer agents may be used in combination of two or more kinds. In this case, the dose of the antibacterial agent is normally 1 to 500 mg, preferably 5 to 200 mg, per adult per day in oral administration; the dose of antiulcer agent is normally 0.5 to 1,000 mg, preferably 1 to 500 mg, per adult per day in oral administration.

Therefore, the medicinal composition comprising Compound (I) according to the invention can be administered as a safe antibacterial agent or as a safe antiulcerative drug to man and other mammals (e.g. human, canine, feline, monkey, rat, mouse, equine, bovine, etc.), alone or together with a pharmaceutically acceptable carrier, either orally or parenterally. Usually, the oral route of administration is preferred.

The dosage form which can be used for oral medication includes but is not limited to tablets (inclusive of dragees and film-coated tablets), pills, granules, fine granules, powders, capsules (inclusive of soft capsules), syrup, emulsion, and suspension. The dosage form for parenteral administration includes but is not limited to injections, infusions, drip infusions, and suppositories.

The oral medication is preferably administered as the gastric mucosa-adhesive composition (the gastric mucosa-adhesive agent).

The gastric mucosa-adhesive composition according to the present invention is, for instance, a composition comprising (a) a compound (I) having anti-Helicobacter pylori activity, (b) a lipid and/or a polyglycerol fatty acid ester and (c) a viscogenic agent (a material which becomes sufficiently viscous with water to attach itself to the gastric mucosa). The composition is at least adapted to attach itself to the gastric mucosa and/or otherwise stay in the stomach and release the active ingredient such as anti-Helicobacter pylori substance contained therein at a suitable rate and thereby display a potentiated pharmaceutical effect (e.g. anti-Helicobacter pylori action). The composition is preferably be a composition further comprising (d) a material which swells a viscogenic agent (e.g. a curdlan and/or a low-substituted hydroxypropylcellulose as a swelling material). Though there is no particular limitation on its dosage form, the composition is preferably a solid composition and particularly a composition containing a matrix. The matrix may, for example, be a gastric mucosa-adhesive matrix comprising (a), (b) a polyglycerol fatty acid ester and (c), or a gastric mucosa-adhesive matrix comprising (a), (b) a lipid and (c). The preferred matrix is a gastric mucosa-adhesive matrix comprising (b) a polyglycerol fatty acid ester. The preferable example of the gastric adhesive composition of the present invention is a composition further comprising (d) a material which swells a viscogenic agent.

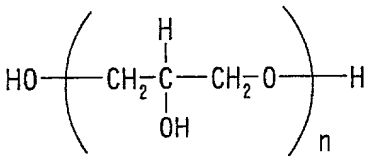
The gastric mucosa-adhesive matrix comprising said four components (a), (b), (c), and/or (d) is preferably a matrix such that the viscogenic agent is dispersed in the matrix which comprises the polyglycerol fatty acid ester or lipid or a matrix which is covered with the viscogenic agent. The melting point of the gastric mucosa-adhesive matrix may, for example, be about 30° to about 120° C and preferably about 40° to about 120° C.

The polyglycerol fatty acid ester for use in the present invention is esters of polyglycerols with fatty acids and may be a mono- or poly-ester (diester, triester, etc.). The polyglycerol fatty acid ester is characterized in that it does not undergo polymorphic transition or any material interaction with the active

ingredient, allowing those coexisting ingredients to remain undeactivated and stable for an extended period of time.

Polyglycerol by definition is "a polyhydric alcohol containing n (cyclic form) to (n+2) (straight-chain form or branched form) hydroxy groups and (n-1) (straight-chain form or branched form) to n (cyclic) ether bonds per molecule" [Polyglycerin Esters, (ed.) Sakamoto Yakuhin Kogyo Co., Ltd., published October 4, 1994], and any straight-chain ester or branched-chain ester can be used in the present invention.

For example, compounds of the following formula (VI) can be employed.



(VI)

(wherein n represents a degree of polymerization which is an integer of not less than 2). The value of n is generally about 2 to about 50, preferably about 2 to about 20, and for still better results, about 2 to about 10.

The polyglycerol includes but is not limited to diglycerol, triglycerol, tetraglycerol, pentaglycerol, hexaglycerol, heptaglycerol, octaglycerol, nonaglycerol, decaglycerol, pentadecaglycerol, eicosaglycerol, and triacontaglycerol. Among those polyglycerols, tetraglycerol, hexaglycerol or decaglycerol is used in many cases.

The fatty acid includes but is not limited to saturated or unsaturated fatty acids each containing about 8 to about 40, preferably about 12 to about 25, and more preferably about 15 to about 22 carbon atoms. The preferred fatty acid is stearic acid, oleic acid, lauric acid, linoleic acid, linolenic acid, ricinoleic acid, caprylic acid, capric acid, or behenic acid.

The polyglycerol fatty acid ester includes but is not limited to behenic acid hexa(tetra)glyceride, caprylic acid mono(deca)glyceride, caprylic acid di(tri)glyceride, capric acid di(tri)glyceride, lauric acid mono(tetra)glyceride, lauric acid mono(hexa)glyceride, lauric acid mono(deca)glyceride, oleic acid

mono(tetra)glyceride, oleic acid mono(hexa)glyceride, oleic acid mono(deca)glyceride, oleic acid di(tri)glyceride, oleic acid di(tetra)glyceride, oleic acid sesqui(deca)glyceride, oleic acid penta(tetra)glyceride, oleic acid penta(hexa)glyceride, oleic acid deca(deca)glyceride, linoleic acid mono(hepta)glyceride, linoleic acid di(tri)glyceride, linoleic acid di(tri)glyceride, linoleic acid di(tetra)glyceride, linoleic acid di(hexa)glyceride, stearic acid mono(di)glyceride, stearic acid mono(tetra)glyceride, stearic acid penta(tetra)glyceride, stearic acid mono(deca)glyceride, stearic acid tri(tetra)glyceride, stearic acid penta(hexa)glyceride, stearic acid tri(hexa)glyceride, stearic acid deca(deca)glyceride, palmitic acid mono(tetra)glyceride, palmitic acid mono(hexa)glyceride, palmitic acid mono(deca)glyceride, palmitic acid tri(tetra)glyceride, palmitic acid tri(hexa)glyceride, palmitic acid sesqui(hexa)glyceride, palmitic acid penta(tetra)glyceride, palmitic acid penta(hexa)glyceride, palmitic acid deca(deca)glyceride, and polyglycerol polyricinolate (e.g. polyglycerol polyricinolate, etc.).

The preferred polyglycerol fatty acid ester includes, for instance, behenic acid hexa(tetra)glyceride (e.g. HB-310™, Sakamoto Yakuhin Kogyo Co., Ltd.,; Poem J-46B™, Riken Vitamin Co.), stearic acid penta(tetra)glyceride (e.g. PS-310™, Sakamoto Yakuhin Kogyo Co., Ltd.), stearic acid mono(tetra)glyceride (e.g. MS-310™, Sakamoto Yakuhin Kogyo Co., Ltd.), stearic acid penta(hexa)glyceride (e.g. PS-500™, Sakamoto Yakuhin Kogyo Co., Ltd.), stearic acid mono(deca)glyceride, polyglycerol polyricinolate (e.g. tetraglycerol polyricinolate, etc.) (e.g. CRS-75™, Sakamoto Yakuhin Co., Ltd.) and mixtures of such glycerides.

Those polyglycerol fatty acid esters can be used each alone or as a mixture of two or more species, preferably about 2 or about 3 species.

The molecular weight of the polyglycerol fatty acid ester is generally about 200 to about 5000, preferably about 300 to about 3000, preferably about 2000 to about 3000. The hydrophile-

lipophile balance (HLB) number of the polyglycerol fatty acid ester is generally about 1 to about 22, preferably about 1 to about 15, more preferably about 1 to about 9, for still better results, about 1 to about 4. Two or more polyglycerol fatty acid esters differing in HLB number from each other may be used in combination to provide for the designed HLB number. By adjusting the HLB of the polyglycerol fatty acid ester judiciously, the release and dissolution kinetics of the active drug substance can be controlled as desired.

The proper polyglycerol fatty acid ester can be selected with reference to the particular active ingredient (e.g. anti-Helicobacter pylori agent, etc.), viscogenic agent, swelling material (e.g. curdlan, and/or low-substituted hydroxypropylcellulose, etc.), the particular combination thereof, and the objective form of the composition. Preferably, however, compounds which are solid at atmospheric temperature (ca 15° C) are employed. The melting point of the polyglycerol fatty acid ester may, for example, be about 15 to about 80° C, preferably about 30 to about 75° C, and for still better results, about 45 to about 75° C.

A suitable polyglycerol fatty acid ester is selected according to the species of active ingredient used and the intended dosage form. Generally, polyglycerols with degrees of polymerization in the range of about 2 to about 16 are preferred. The particularly preferred range is about 2 to about 10. Preferred are esters such that the fatty acid has formed an ester bond with at least one of the (degree of polymerization +2) hydroxy groups, preferably such that the fatty acid or acids have formed ester bonds with not less than about 60%, more preferably not less than about 80%, of the total number of hydroxy groups in the polyglycerol. The fatty acid or acids are preferably saturated acids each containing about 6 to about 22, more preferably about 15 to about 25, and for still better result, about 18 to about 22 carbon, atoms. The fatty acid involved in the formation of the ester bonds may be of the same kind or different kinds.

In the production of a solid composition according to the present invention by using two or more different polyglycerol fatty

acid esters as a mixture, a liquid polyglycerin fatty acid ester may be included in the mixture as long as the final composition is solid at atmospheric temperature.

When the polyglycerol fatty acid ester is used as a gastric mucosa-adhesive matrix, the amount of the polyglycerol fatty acid ester relative to the total weight of the composition is generally about 5 to about 98 weight %, preferably about 20 to about 95%, more preferably about 40 to about 95% and to the active ingredient in the composition may, for example, be about 0.01 to about 15000 times by weight, preferably about 0.1 to about 1000 times by weight, and for still better result, about 0.1 to about 100 times by weight.

The lipid for use in the present invention is one having a melting point of about 40 to about 120° C, preferably about 40 to about 90° C.

The lipid includes but is not limited to saturated fatty acids of about 14 to about 22 carbon atoms (e.g. myristic acid, stearic acid, palmitic acid, behenic acid, etc.) or salts (sodium salt, potassium salt, etc.) thereof; higher alcohols of about 16 to about 22 carbon atoms (e.g. cetyl alcohol, stearyl alcohol, etc.); fatty acid glycerol esters such as the monoglycerides, diglycerides, triglycerides, etc. of the above-mentioned fatty acids (e.g. 1-monostearin, 1-monopalmitin, etc.); oils (e.g. castor oil, cottonseed oil, beef tallow, etc., inclusive of the corresponding hydrogenated oils); waxes (e.g. beeswax, carnauba wax, sperm wax, etc.); hydrocarbons (e.g. paraffin, microcrystalline wax, etc.); and phospholipids (e.g. hydrogenated lecithin etc.). Among those lipids, oils, waxes, C_{14-22} saturated fatty acids, C_{16-22} higher alcohols, and hydrocarbons are preferred. The more preferred are hydrogenated cottonseed oil, hydrogenated castor oil, hydrogenated soybean oil, carnauba wax, stearic acid, stearyl alcohol, and microcrystalline wax. The most preferred is hydrogenated castor oil or carnauba wax.

When a lipid is used as the gastric mucosa-adhesive matrix, the amount of the lipid relative to the total weight of the composition is generally about 5 to about 98 weight %, preferably about 20 to about 95 weight %, more preferably about 40 to about

95 weight %, and to the active ingredient in the composition is about 0.01 to about 15000 times by weight, preferably about 0.1 to about 1000 times by weight, and for still better result, about 0.1 to about 100 times by weight.

The above-mentioned polyglycerol fatty acid ester and lipid may be used as a mixture. For example, the combination of a polyglycerol fatty acid ester with a wax or the combination of a polyglycerol fatty acid ester with a hydrogenated oil can be mentioned. Specifically, a mixture of 2, 3 or more members selected from among behenic acid hexa(tetra)glyceride, stearic acid penta(tetra)glyceride, stearic acid penta(hexa)glyceride, polyglycerol polyricinolate (e.g. tetraglycerol polyricinolate, etc.), carnauba wax, hydrogenated castor oil, and microcrystalline wax and polyglycerol polyricinolate (e.g. tetraglycerol polyricinolate), can be mentioned.

When the gastric mucosa-adhesive matrix comprising a viscogenic agent in addition to said polyglycerol fatty acid ester and/or lipid is used for the composition of the invention, the total amount of the polyglycerol fatty acid ester and lipid relative to the total weight of the composition is generally about 5 to about 98 weight %, preferably about 20 to about 95 weight %, more preferably about 40 to about 95 weight %, and to the active ingredient in the composition is about 0.01 to about 15000 times by weight, preferably about 0.1 to about 1000 times by weight, and for still better result, about 0.1 to about 100 times by weight.

A lipid may be incorporated in a matrix comprising the polyglycerol fatty acid ester. The lipid is a pharmaceutically acceptable water-insoluble substance capable of regulating the dissolution kinetics of the active ingredient. The lipid includes those species mentioned hereinbefore.

When a lipid and a polyglycerol fatty acid ester are used in combination, the amounts of the lipid and polyglycerol fatty acid ester need only be within the range not detracting from the adhesion to the gastrointestinal mucosa and can be selected from said range of total amount, and the amount of the lipid relative to the polyglycerol fatty acid ester may be about 0.01 to about 1000 times

by weight, preferably about 0.1 to about 200 times by weight, and for still better results, about 0.1 to about 100 times by weight.

The swelling material used in the present invention is a material which swells a viscogenic agent or accelerates the swell of a viscogenic agent caused by water.

Any type of swelling material can be used in the present invention as long as it has the characteristics described above and is pharmaceutically acceptable. For instance, preferably a curdlan and/or a low-substituted hydroxypropylcellulose can be used.

The amount of the swelling material in the gastric mucosa-adhesive composition of the present invention is about 0.5 to about 50 weight %, preferably about 1 to about 40 weight %, and for still better results, about 1 to about 30 weight %, relative to the total weight of the composition.

The curdlan for use in the present invention is a linear water-insoluble polysaccharide (b-1,3-glucan) produced by microorganisms (such as Alcaligenes faecalis var. myxogenes etc.), which includes such species as curdlan 10C3K, 13140, 12607, 12665, 13127, 13256, 13259, and 13660 [New Food Industry, 20, No. 10, p. 49 (1978)]. Among those and other species of curdlan, those which are acceptable as pharmaceutical bases or excipients can be employed. A preferred example is curdlan N (a food additive).

The amount of the curdlan in the gastric mucosa-adhesive composition of the invention relative to the total weight of the composition is about 0.5 to about 50 weight %, preferably about 1 to about 40 weight %, and more preferably about 1 to about 30 weight %.

The low-substituted hydroxypropylcellulose for use in the present invention is a cellulose derivative available upon substitution of hydroxypropoxy for some of the hydroxy groups of cellulose, which has a hydroxypropoxy content of 5.0 to 16.0% (as specified in the Japanese Pharmacopoeia Twelfth Edition). The low-substituted hydroxypropyl cellulose mentioned above is useful, in particular, one which has a hydroxypropoxy content of 7.0 to 13.0% (e.g. L-HPC™, Shin-Etsu Chemicals., Co., Ltd. is preferred. Thus, those derivatives with a degree of substitution within the above

range and varying in particle diameter, such as LH-11™ (Shin-Etsu Chemicals., Co., Ltd. hydroxypropoxy content 10.0 to 12.9%, particle size distribution ≧98% under 150 μm sieve and ≦0.5% on 180 μm sieve), LH-20™ (Shin-Etsu Chemicals., Co., Ltd., hydroxypropoxyl content 13.0-16.0%, particle size distribution ≧ 90% under 75 μm sieve and ≦1.0% on 106 μm sieve), LH-21 (Shin-Etsu Chemicals., Co., Ltd., hydroxypropoxyl content 10.0 to 12.9%, particle size distribution ≧90% under 75mm sieve and ≦1.0% on 106 μm sieve), LH-22 (Shin-Etsu Chemicals., Co., Ltd., hydroxypropoxyl content 7.0 to 9.9%, particle size distribution ≧90% under 75 μm sieve and ≦1.0% on 106 μm sieve), and LH-31 (Shin-Etsu Chemicals., Co., Ltd., hydroxypropoxyl content 10.0 to 12.9%, mean particle diameter not greater than 30 μm, particle size distribution ≧50% under 45 mm sieve and ≦5.0% on 75 mm sieve), among others, can be utilized.

Preferably, LH-22 or LH-31 is utilized.

The amount of the low-substituted hydroxypropylcellulose in the gastric mucosa adhesive composition of the present invention is about 0.5 to about 50 weight %, preferably about 1 to about 40 weight %, and for still better results, about 1 to about 30 weight %, relative to the total weight of the composition.

Any type of viscogenic agent can be used in the present invention as long as it becomes sufficiently viscous with water to attach itself to the gastrointestinal mucosa and is pharmaceutically acceptable. Preferred, however, are those substances which are markedly swollen by water and develop high degrees of viscosity. The viscogenic agent, thus, includes synthetic polymers and naturally-occurring viscogenic materials.

The preferred synthetic polymer is a polymer such that the viscosity of a 2% aqueous solution thereof at 20° C is about 3 to about 50000 cps., preferably about 10 to about 30000 cps., and for still better results, about 15 to about 30000 cps. However, when a basic or an acidic polymer which gains in viscosity on neutralization is used, the preferred polymer is such that the

viscosity of a 0.2% solution thereof after neutralization at 20° C is about 100 to about 500000 cps, preferably about 100 to about 200000 cps, and for still better results, about 1500 to about 100000 cps.

The value of the viscosity is measured with a Brookfield viscometer at about 20° C.

Preferably the above-mentioned polymer is an acidic polymer which includes but is not limited to carboxyl-or sulfo-containing polymers and the corresponding salt-containing polymers. Particularly preferred are carboxyl-containing polymers and carboxylate salt-containing polymers.

The carboxyl (inclusive of its salt)-containing polymer is preferably an acrylic homopolymer or copolymer containing acrylic acid as a monomer unit or its salt. The salt includes monovalent metal salts such as the sodium salt, potassium salt, etc., divalent metal salts such as the magnesium salt, calcium salt, etc., ammonium salt, etc.

The acrylic polymer, inclusive of its salt, includes polymers containing carboxyl groups in a proportion of about 58 to about 63 weight % and having a molecular weight of about 20×10^4 to about 600×10^4, preferably about 100×10^4 to about 600×10^4, and more preferably about 100×10^4 to about 500×10^4. The preferred acrylic polymer, inclusive of its salt, includes acrylic acid homopolymers and their salts. Such polymers are listed under the heading of carboxyvinyl polymer in Japanese Standards of Pharmaceutical Ingredients (October 1986).

As specific examples of said acrylic polymer, there can be mentioned carbomer [Carbopol™ (hereinafter referred to as Carbopol), The B.F. Goodrich Company] 940, 934, 934P, 941, 1342, 974P, 971P (NF XVIII), EX214 etc., HIVISWAKO™ 103, 104, 105, and 204 (Wako Pure Chemical Industries), NOVEON AA1™ (The B.F. Goodrich Company), and calcium polycarbophil (US Patent XXIII)).

The naturally-occurring viscogenic agent includes but is not limited to mucin, agar, gelatin, pectin, carrageenin, sodium alginate, locust bean gum, xanthan gum, tragacanth gum, chitosan, pullulan, waxy starch, sucralfate, curdlan, and cellulose and its

derivatives (e.g. cellulose sulfate) and preferably hydroxypropylcellulose, hydroxypropylmethylcellulose, etc.).

The most preferred viscogenic agent is an acrylic polymer or its salt.

Those viscogenic agents can be used alone or in combination.

Referring to the amount of the viscogenic agent for use in the composition of the invention, its amount in the gastric mucosa adhesive matrix may for example be about 0.005 to about 99 weight %, preferably about 0.5 to about 45 weight %, more preferably about 1 to about 30 weight %, furthermore preferably about 1 to about 25 weight %, and for still better result, about 1 to about 20 weight %. When, for example, the viscogenic agent is dispersed in a matrix comprising the polyglycerol fatty acid ester and/or lipid, the amount of the viscogenic agent is about 0.005 to about 95 weight %, preferably about 0.5 to about 30 weight %, and more preferably about 1 to about 25 weight %, and for still better result, about 1 to about 20 weight % based on the total weight. When the matrix is coated with the viscogenic agent, the proportion of the viscogenic agent is also about 0.005 to about 95 weight %, preferably about 0.5 to about 30 weight %, and more preferably about 1 to about 25 weight %, and for still better result, about 1 to about 20 weight based on the total weight.

When the composition of the present invention contains a curdlan as a swelling material, the composition is capable of attaching itself to the gastrointestinal mucosa even without addition of said viscogenic agent, for the curdlan acts as a viscogenic agent by itself. In this case, the curdlan may be formulated in an amount beyond the range defined hereinbefore for imparting the necessary adherent effect.

The gastric mucosa adhesive composition comprising the viscogenic agent dispersed in a matrix comprising a polyglycerol fatty acid ester and/or lipid may be any dispersion of the polyglycerol fatty acid ester and/or lipid, viscogenic agent, curdlan and/or low-substituted hydroxypropylcellulose, and active ingredient. Dispersion can be effected by the analogue to the <u>per se</u> known technology.

The amount of Compound (I) in the medicinal composition of the invention is generally 2 to 85 weight % and preferably 5 to 70 weight %.

The manufacturing technology for the pharmaceutical composition (especially, the gastric mucoa adhesive composition)comprising the compound (I) of the present invention include those known methods which are in common usage in the pharmaceutical field. Moreover, the composition can be manufactured using suitable amounts of the additives (e.g. dilutions, excipient, binder, disintegrator, lubricant, sweetener, surfactant, suspending agent, emulsifier, etc.) which are generally used in the pharmaceutical industry.

For the manufacture of tablets containing Compound (I), for instance, said excipient, binder, disintegrator, and lubricant are employed. For the manufacture of pills or granules or fine granules, the excipient, binder, and disintegrator are formulated. The excipient is also used in the manufacture of powders or capsules, while the sweetener is added in the manufacture of a syrup. In the manufacture of an emulsion or a suspension, the suspending agent, surfactant, and/or emulsifier is added. The excipient includes but is not limited to lactose, sucrose, glucose, starch, cane sugar, microcrystalline cellulose, licorice powder, mannitol, sodium hydrogencarbonate, calcium phosphate, and calcium sulfate. The binder includes but is not limited to 5 to 10 wt. % starch solution, 10 to 20 wt. % gum arabic solution or gelatin solution, 1 to 5 wt. % gum tragacanth solution, carboxymethylcellulose solution, sodium alginate solution, and glycerin. The disintegrator includes but is not limited to starch and calcium carbonate. The lubricant includes but is not limited to magnesium stearate, stearic acid, calcium stearate, and purified talc. The sweetener includes but is not limited to glucose, fructose, inverted sugar, sorbitol, xylitol, glycerin, and simple syrup. The surfactant includes but is not limited to sodium lauryl sulfate, polysorbate 80, sorbitan fatty acid monoesters, and polyoxyl stearate 40. The suspending agent includes but is not limited to gum arabic, sodium alginate, carboxymethylcellulose sodium, methylcellulose, and bentonite.

The emulsifier includes but is not limited to gum arabic, gum tragacanth, gelatin, and polysorbate 80. Aside from the above, the colorant, preservative, flavorant, corrigent, stabilizer, thickener, and other common additives for pharmaceutical use can be formulated in suitable amounts in the manufacture of said dosage forms containing Compound (I).

The example of the technology for production of a gastric mucosa adhesive composition of the present invention is now described.

1) The gastric mucosa adhesive composition, which is solid at atomospheric temperature, can be produced in a similar manner to the per se known technology. A typical process comprises melting the polyglycerol fatty acid ester and/or lipid at a temperature beyond its melting point, adding said viscogenic agent, anti-Helicobacter pylori agent, and curdlan and/or low-substituted hydroxypropylcellulose either at one time or serially to the melt to thereby disperse them in the melt, and cooling the dispersion. The heating temperature may for example be about 40 to about 150° C, preferably about 50 to about 110° C, and more preferably about 50 to about 100° C. This process can be carried out with a conventional granulating machine and the composition is preferably molded into solid beads (e.g. granules, fine granules, etc.) by spray cooling, for example spray chilling.

The spray chilling method may typically comprise dripping a mixed dispersion of the viscogenic agent, curdlan and/or low-substituted hydroxypropylcellulose, and active ingredient in a molten polyglycerol fatty acid ester and/or lipid at a constant flow rate onto a rotary disk revolving at a high speed of, for example, about 10 to about 6000 rpm, preferably about 900 to about 6000 rpm, and more preferably about 1000 to about 5000 rpm. The rotary disk may for example be a flat, smooth disk, typically made of aluminum and measuring about 5 to about 100 cm in diameter, preferably about 10 to about 20 cm in diameter. The dripping rate of said molten dispersion can be selected according to the designed particle diameter and is generally about 1 to about 1000 g/min., preferably about 2 to about 200 g/min., more preferably about 5 to about 100 g/min. The granules thus obtained are true to spheres so that a

uniform film can be formed on their surface with good efficiency in the subsequent coating step.

An alternative production process comprises kneading the viscogenic agent, curdlan and/or low-substituted hydroxypropylcellulose, and active ingredient into the polyglycerol fatty acid ester and/or lipid and granulating the resulting dispersion. The solvent for use in this process may be a solvent of the common variety (e.g. methanol, acetonitrile, chloroform, etc.).

A further alternative process for producing the solid composition comprises the use of the melt granulation technology. A typical melt granulation process comprises heating the polyglycerol fatty acid ester and/or lipid at a temperature near its melting point, for example, a temperature from its melting point to a temperature about 5° C below the melting point, subjecting the resulting melt to granulation, such as the above-mentioned spray chilling, and suspending the resulting fine particles together with the viscogenic agent, anti-Helicobacter pylori agent, and curdlan and/or low-substituted hydroxypropylcellulose under heating at a suitable temperature to provide an adhesive matrix-drug system. In this case, the influence of heat on the active ingredient can be avoided.

The solid composition comprising a matrix made up of a polyglycerol fatty acid ester and/or a lipid and coated with a viscogenic agent may be a preparation coated with such a viscogenic agent alone or a mixture of a viscogenic agent and a swelling material (e.g. curdlan and/or a low-substituted hydroxypropylcellulose etc), preferably with a coating material containing either a viscogenic agent alone or a viscogenic agent plus a curdlan and/or a low-substituted hydroxypropylcellulose. The coating material may be a composition containing at least one member selected from among said polyglycerol fatty acid ester, said lipid, and said water-insoluble polymer. When a viscogenic agent which is sparingly compatible or incompatible with the components of the solid composition is employed for coating, the solid composition can be provided with a film in which the viscogenic agent has been dispersed.

The coating material may further contain the additives mentioned hereinbefore.

The water-insoluble (hydrophobic) polymer includes but is not limited to hydroxypropylmethylcellulose phthalate (The Japanese Pharmacopoeia Twelfth Edition), hydroxypropylmethylcellulose acetate succinate (Shin-Etsu Chemicals Co., Ltd.), carboxymethylethylcellulose (Freund Industries Co., Ltd., CMEC, Japanese Standards of Pharmaceutical Ingredients, 1986), cellulose acetate trimellitate (Eastman), cellulose acetate phthalate (The Japanese Pharmacopoeia Twelfth Edition), ethylcellulose (Asahi Chemical Industry Co., Ltd.), aminoalkyl methacrylate copolymer (Röhm-Pharma, Eudragit™ RS-100, RL-100, RL-PO, RS-PO, RS-30D, RL-30D), methacrylic acid-ethyl acrylate copolymer (Röhm-Pharma, Eudragit™ L100-55), methacrylic acid-methyl methacrylate copolymer (Röhm-Pharma, Eudragit™ L-100, S-100), Eudragit™ L30D-55, Eudragit™ NE-30D (Röhm-Pharma), and polyvinyl acetate (Colorcon). Those hydrophobic polymers can be used independently or as a mixture of two or more different polymers.

The proportion of the viscogenic agent in the coating material is about 0.005 to about 100 weight %, preferably about 0.05 to about 95 weight %, more preferably about 0.05 to about 30 weight %, and for still better result, about 1 to about 10 weight % based on the whole solid fraction of the coating material.

When at least one of the polyglycerol fatty acid ester, lipid, and hydrophobic polymer is used in combination with the viscogenic agent for the coating material, the proportion of the viscogenic agent based on the total weight of the solid fraction of the coating material is about 0.05 to about 95 weight %, preferably about 0.5 to about 95 weight %, more preferably about 0.5 to about 30 weight %, futhermore preferably about 5 to about 30 weight %, and for still better result, about 5 to about 25 weight %.

Referring further to the coating material, two or more members selected from the class consisting of the polyglycerol fatty acid ester, lipid, and hydrophobic polymer can be used in combination. In this case, based on each part by weight of the whole polyglycerol fatty acid ester and/or lipid, the remaining component is used in

a proportion of about 0.0001 to about 1000 part by weight, preferably about 0.01 to about 100 part by weight, and more preferably about 0.01 to about 10 part by weight.

The coating amount can be selected according to the type of solid composition and the desired strength of adhesion to the mucosa. For example, the coating amount for a solid composition may be about 0.1 to about 30 weight %, preferably about 0.5 to about 20 weight %, for tablets and about 0.1 to about 100 weight %, preferably about 1 to about 50 weight %, for fine granules.

Where necessary, the coating material may be supplemented with the common additives such as those mentioned hereinbefore. For example, the coating material and the additive may be added together or separately, etc. applied. The proportion of the additive relative to the solid fraction of the coating material is about 0.1 to about 70 weight %, preferably about 1 to about 50 weight %, and more preferably about 20 to about 50 weight %.

The coating technology that can be used includes a variety of per se known methods, such as pan coating, fluidized-bed coating, roll coating, etc. When the coating material is a solution or dispersion containing water or an organic solvent, the spray coating method can also be employed. There is no particular limitation on the kind of said water or organic solvent. Thus, for example, alcohols such as methanol, ethanol, isopropyl alcohol, etc.; ketones such as acetone etc.; and halogenated hydrocarbons such as chloroform, dichloromethane, trichloromethane, etc. can be used.

When the polyglycerol fatty acid ester and/or lipid is used for coating, the objective coated composition can be produced by melting the polyglycerol fatty acid ester and/or lipid, optionally together with other additives, under heating, emulsifying the melt with water, spray-coating the surface of a solid composition with the resulting emulsion, and drying the coat. An alternative procedure comprises adding the coating material to the solid composition preheated in a coating pan or the like and melt-spreading the coating.

The solid composition is coated generally at a temperature of about 25 to about 60° C and preferably at about 25 to about 40° C.

The coating time can be judiciously selected with reference to the coating method, the characteristics and amount of the coating material, and characteristics of the substrate solid composition.

Insofar as a sufficient adhesion to the gastrointestinal mucosa can be assured, the gastric mucosa adhesive solid composition may, if necessary, be further coated with a conventional gastric coating agent or a water-soluble coating agent.

The gastric mucosa adhesive composition according to the present invention can generally be administered orally as it is or in a suitable preparation. The solid oral dosage form includes but is not limited to fine granules, granules, pills, tablets manufactured by compressing said fine granules or granules with a tablet machine, and capsules manufactured by filling said fine granules or granules into suitable capsule shells. Among those preparations, fine granules and granules are preferred.

The particle size distribution of said fine granules may for example be : particles measuring about 10 to about 500 μm in diameter account for not less than about 75 weight %, particles larger than about 500 μm account for not more than about 5 weight %, and particles smaller than about 10 μm account for not more than about 10 weight %. The preferred distribution is about 105 to about 500 μm accounting for about $\geqq$75 weight %, about $\geqq$500 μm accounting for not more than about 5 weight %, and about $\leqq$74 μm accounting for not more than about 10 weight %. The particle size distribution of said granules may for example be about 500 to about 1410 μm accounting for not less than about 90 weight % and about $\leqq$177 μm accounting for not more than about 5 weight %.

2) When the gastric mucosa adhesive composition is to be provided as a liquid composition, such a liquid composition can be manufactured by the manner similar to the per se known technology. A typical procedure comprises mixing a polyglycerol fatty acid ester and/or a lipid, which is liquid at atmospheric temperature, a viscogenic agent, an active ingredient, and a swelling material (e.g. a curdlan and/or a low-substituted hydroxypropylcellulose etc.) all at once or serially to provide a dispersion or solution.

The dosage form comprising such a liquid adherent mucosal medication system includes but is not limited to syrups, emulsions, suspensions, and encapsulated versions thereof.

The proportion of the active ingredient (e.g. an anti-HP agent etc.) in the composition of the invention is about 0.005 to about 95 weight %, preferably about 1 to about 95 weight %, and more preferably about 10 to about 95 weight %, and for still better result, about 10 to about 50.

The medicinal composition (especially, a gastric mucosa adhesive composition) of the present invention comprising Compound (I) or its prodrug is stable and less toxic and can therefore be used safely. The daily oral dosage, which depends on the patient's clinical status and body weight, the particular species of compound, and the route of administration, for an adult patient (body weight: ca 60 kg), for example, with gastric ulcer associated with Helicobacter pylori infection is 1 to 500 mg, preferably about 10 to 200 mg, as the active ingredient (Compound (I) or its prodrug).

BEST MODE FOR CARRYING OUT THE INVENTION

The following reference examples, examples, experimental examples, and formulation examples are only intended to illustrate the present invention in further detail and should by no means be construed as defining the scope of the invention. The NMR spectra were those recorded using Bruker AC-300 Spectrometer or Varian gemini 200 Spectrometer. All the δ values are shown in ppm unit, and the meanings of the following abbreviated symbols are as follows. s : singlet, d : doublet, t : triplet, dd : double doublet, m : multiplet. Room temperature means 15~25 ℃ but is not limited strictly.

Reference Example 1

(*S*)-3-[(2*S*,3*R*,4*R*,5*S*)-2,3,4,6-tetrahydroxy-5-(L-valyl-L-leucyl)aminohexanoyl]amino-3-phenylpropionic acid (HC-70II, compound 2) and (*S*)-3-[(2*S*,3*R*,4*R*,5*S*)-2,3,4,6-tetrahydroxy-5-(L-leucyl)aminohexanoyl]amino-3-phenylpropionic acid (HC-70III)

A loopful of Bacillus sp. HC-70 (The accession number:IFO-16098(Institute for Fermentation, Osaka)) sufficiently grown on a

slant medium composed of glucose 0.1%, tryptone 0.5%, yeast extract 0.25%, and agar 1.5% was used to inoculate a 2-L Sakaguchi flask containing 500 mL of a seed culture medium (pH 7.0) composed of glucose 2.0%, soluble starch 3.0%, corn steep liquor 0.3%, soybean flour 1.0%, polypeptone 0.5%, yeast extract 0.1%, oatmeal agar 0.2%, sodium chloride 0.3%, and precipitated calcium carbonate 0.5% and incubation was carried out on a reciprocating shaker at 24° C for 2 days. The culture, 500 mL, was transferred to a 200-L fermentor containing 120 L of a production medium (pH 6.5) composed of glucose 0.5%, dextrin 5.0%, soybean meal 3.5%, yeast extract 0.5%, precipitated calcium carbonate 0.7%, ACTOCOL™ 31-56 (Takeda Chemical Industries Ltd.) 0.05%, and silicone oil 0.05% and fermention was carried out at a temperature of 22° C and an internal pressure of 1.0 kg/cm^2 under 120 L/min. aeration and 120 rpm agitation for 42 hours.

The resulting culture broth (120 L) was adjusted to pH 7 and filtered with a filter aid (Radiolite 600, Showa Chemical Industry). The filtrate (130 L) was adjusted to pH 7 and subjected to HP-20 (7 L) column chromatography. After the column was washed with water (21 L), elution was carried out with 30% (v/v) isopropyl alcohol/H_2O (28 L). The eluate was concentrated and the residue was diluted with water to a volume of 30 L and subjected to CNP-80 (H-form, 15 L) column chromatography. After the column was washed with water (45 L), elution was carried out with 2N-aqueous ammonia (53 L). The eluate was concentrated and subjected to PA-412 (OH-form, 2 L) column chromatography. The column was washed with water (6 L) and 1 M sodium chloride/H_2O (2 L) in that order and serial elution was carried out with 1 M sodium chloride/H_2O (10 L) and 1N-hydrochloric acid (4 L). The eluate was adjusted to pH 7 and subjected to HP-20 (1 L) column chromatography. The column was washed with water (3 L) and elution was carried out with 30% (v/v) isopropyl alcohol/H_2O (3.4 L). The eluate was concentrated, adjusted to pH 7, and subjected to HP-20S (400 mL) column chromatography. After the column was washed with water (1.2 L), serial elution was carried out with 5% (v/v) isopropyl alcohol/H_2O (1.2 L) and 10% (v/v) isopropyl alcohol/H_2O (1.2 L). The 5% (v/v) isopropyl alcohol/H_2O

eluate was concentrated and subjected to HP-20SS (100 mL) column chromatography. This column was washed with water (200 mL) and serial elution was carried out using water (100 mL), 2% (v/v) isopropyl alcohol/H_2O (300 mL), and 5% (v/v) isopropyl alcohol/H_2O (300 mL). The eluate was concentrated and allowed to stand at 7° C and the crystal crop was harvested to provide HC-70III (1.3 g). The 10% (v/v) isopropyl alcohol/H_2O eluate from the HP-20S (400 mL) column was concentrated, and after addition of methanol, the concentrate was allowed to stand at 7° C and the resulting crystals (1.7 g) were collected by filtration. This crystal crop was recrystallized twice from water. In this manner, a crystal crop (1.3 g) composed predominantly of HC-70II was obtained. Of this crystal crop, 719 mg was subjected to HP-20S (70 mL) column chromatography. The column was washed with water (210 mL), 2% (v/v) isopropyl alcohol/H_2O (210 mL), and 5% (v/v) isopropyl alcohol/H_2O (210 mL), and elution was carried out with 10% (v/v) isopropyl alcohol/H_2O (420 mL). The HC-70II fraction was concentrated and allowed to stand at 7° C and the resulting crystals were recovered by filtration to provide HC-70II (479 mg).

Reference Example 2

(Acquisition of HC-70III by using Bacillus insolitus HC-72[the accession number: IFO-16179(Institute for Fermentation, Osaka), FERM BP-6385(National Institute of Bioscience and Human Technology, Japan)])

A loopful of Bacillus insolitus HC-72 sufficiently grown on a slant medium composed of glucose 0.1%, tryptone 0.5%, yeast extract 0.25%, and agar 1.5% was used to inoculate a 2 L Sakaguchi flask containing 500 mL of a seed culture medium (pH 7.0) composed of glucose 2.0%, soluble starch 3.0%, corn steep liquor 0.3%, soybean flour 1.0%, polypeptone 0.5%, yeast extract 0.1%, sodium chloride 0.3%, and precipitated calcium carbonate 0.5% and incubation was carried out on a reciprocating shaker at 28° C for 1 day. The culture, 500 mL, was transferred to a 200-L fermentor containing 120 L of a production medium (pH 7.0) composed of glucose 2.0%, soluble starch 3.0%, corn steep liquor 0.3%, soybean flour 1.0%, polypeptone 0.5%, yeast extract 0.1%, sodium chloride 0.3%,

precipitated calcium carbonate 0.5%, ACTOCOL™ 31-56 (Takeda Chemical Industries Ltd.) 0.05%, and silicone oil 0.05% and incubated at a temperature of 24° C and an internal pressure of 1.0 kg/cm^2 under 120 L/min. aeration and 120 rpm agitation for 48 hours. The culture, 10 L, was transferred to a 2000-L fermentor containing 1200 L of a production medium (pH 7.0) composed of glucose 0.5%, myo-inositol 1.0%, soybean meal 5.0%, corn steep liquor 1.0%, ACTOCOL™ 31-56 (Takeda Chemical Industries Ltd.) 0.05%, and silicone oil 0.05% and incubated at a temperature of 28° C and an internal pressure of 1.0 kg/cm^2 under 840 L/min. aeration and 30 rpm agitation for 114 hours.

The fermentation broth (1200 L) thus obtained was adjusted to pH 5 and a flocculating agent [0.5 (w/v) Sanfloc C-109P, Sanyo Chemical Industries, Ltd.] was added for flocculation. The broth was then filtered with a filter aid (Radiolite 600). The filtrate (1200 L) was adjusted to pH 5 and subjected to charcoal [Granular Shirasagi (Takeda Chemical Industries Ltd.), 25 L] and SP-850 (100 L) column chromatographies, followed by washing with water (300 L). The SP-850 column alone was serially washed with 0.1N-sodium hydroxide/H_2O (300 L), water (300 L), 0.1N-sulfuric acid (300 L), and water (300 L), and elution was carried out with 25% (v/v) isopropyl alcohol/H_2O (400 L). The HC-70III fraction was adjusted to pH 4.5 and subjected to UBK-510L (Na-form, 150 L) column chromatography. After the column was washed with water (150 L), fractional elution was carried out with 0.01N-aqueous ammonia (600 L). The HC-70III fraction was adjusted to pH 8 and passed columnwise over PK-216 (Na-form, 25 L) and IRA-67 (CH_3COO-form, 25 L) in that order, followed by washing with water (100 L). The effluent and washes were combined, adjusted to pH 5, concentrated, and allowed to stand at 7° C. The resulting crystal crop was harvested by filtration to provide HC-70III (380 g).

Reference example 3

(*S*)-3-[(2*S*,3*R*,4*R*,5*S*)-5-Amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

In phosphate buffer (40 mM, pH 8; 47.5 mL) was dissolved HC-70III (190 mg) followed by addition of an aqueous solution of cobalt

chloride (1 M, 0.19 mL) and Actinase E (19 mg, Kaken Pharmaceutical Co.), and the reaction was carried out at 37°C for 2 hours. This reaction mixture was filtered through a filter paper (No. 2, Toyo Roshi) and the filtrate was subjected to HP-20 (50 mL) column chromatography. The column was washed with water (50 mL) and serial elution was carried out with water (100 mL) and 20% (v/v) isopropyl alcohol/H_2O (200 mL). The eluate was concentrated and freeze-dried to provide crude powders (149 mg).

The above crude powders were subjected to preparative HPLC [column: YMC-Pack SH-363-15, ODS (YMC), mobile phase: 5% (v/v) acetonitrile/0.02 M phosphate buffer (pH 4.5), flow rate: 12 mL/min]. The 400 to 600 mL fractions were pooled, adjusted to pH 7, and concentrated to 120 mL under reduced pressure. The concentrate was chromatographed on an HP-20 (60 mL) column, and after the column was washed with water (180 mL), elution was carried out with 20% (v/v) isopropyl alcohol/H_2O (240 mL). The eluate was concentrated and freeze-dried to provide (*S*)-3-[(2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid as white powders (103 mg). ^{13}C-NMR (DMSO-d_6, d ppm): 174.9, 172.3, 143.4, 127.9, 126.3, 126.2, 71.4, 70.8, 66.6, 60.9, 53.3, 49.7, 43.1. Elemental analysis (for $C_{15}H_{22}N_2O_7 \times 1.5H_2O$) Found : C, 49.11; H, 6.78; N, 7.89. Calcd.: C, 48.78; H, 6.82; N, 7.58.

Reference example 4

(S)-3-[(2S,3R,4R,5S)-5-Amino-2,3,4,6-tetrahydroxyhexanoyl]-amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride

(S)-3-[(2S,3R,4R,5S)-5-Amino-2,3,4,6-tetrahydroxy-hexanoyl]amino-3-phenylpropionic acid (3.40g) was dissolved in 1N hydrochloric acid (11ml). The above solution was concentrated with methanol (10ml). The residue was dissolved in methanol (100ml). To the above solution was added diphenyldiazomethane (3.88g) at room temperature. The reaction mixture was stirred at room temperature for 1.5 hours and concentrated. The residue was washed with diethyl ether to give the title compound (5.36g). ^{1}H-NMR (DMSO-d_6) δ : 3.07 (2H, d, J = 7.0 Hz), 3.50-5.80 (7H, m), 6.68 (1H, s), 7.10-7.20 (15H, m).

Reference example 5

(S)-3-[(2S,3R,4R,5S)-5-[O-tert-Butyl-N-(9-fluorenylmethoxy-carbonyl)-L-seryl]amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of O-tert-butyl-N-(9-fluorenylmethoxycarbonyl)serine (575mg) in acetonitrile (7.5ml) was added N-hydroxysuccinimide (173mg) and dicyclohexylcarbodiimide (309mg). The reaction mixture was stirred at room temperature for 3 hours, filtered and concentrated. The residue was dissolved in dimethylformamide (5ml). To the above solution was added (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (818mg) and triethylamine (0.209ml). The reaction mixture was stirred at room temperature for 24 hours. The reaction was quenched with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized (ether-hexane) to give the title compound (1.156g). ^{1}H-NMR (DMSO-d_6) δ : 1.19 (9H, s), 2.80-6.00 (15H, m), 6.80 (1H, s), 7.05-8.20 (23H, m).

Reference example 6

(S)-3-[(2S,3R,4R,5S)-5-(O-tert-Butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

A mixture of (S)-3-[(2S,3R,4R,5S)-5-[O-tert-butyl-N-(9-fluorenylmethoxycarbonyl)-L-seryl]amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (874mg) and piperidine (5ml) was stirred at room temperature for 5 hours and concentrated. The residue was purified by silica gel column chromatography (eluted with a solution of methanol : ethyl acetate = 1 : 2) and recrystallized (ether-hexane) to give the title compound (593mg). ^{1}H-NMR (DMSO-d_6) δ : 1.12 (9H, s), 3.05 (2H, d, J = 7.0 Hz), 3.20-5.40 (10H, m), 6.67 (1H, s), 7.10-7.40 (15H, m).

Reference example 7

Benzyloxycarbonyl-L-norvalyl-L-asparagine

To a stirred solution of benzyloxycarbonyl-L-norvaline (3g) in acetonitrile (20ml) was added N-hydroxysuccinimide (1.51g) and dicyclohexylcarbodiimide (2.58g). The reaction mixture was stirred at room temperature for 2 hours, filtered and concentrated. The residue was dissolved in ethanol (20ml). The solution was added to a stirred solution of L-asparagine (2.69g) and sodium hydrogen carbonate (1.5g) in water (10ml). The reaction mixture was stirred at room temperature for 2 hours. The reaction was quenched with 1N hydrochloric acid (50ml) and concentrated. The residue was washed with water to give the title compound (3.70g). ^{1}H-NMR (CD_3OD) δ: 0.93 (3H, t, J = 7.2 Hz), 1.30-1.47 (2H, m), 1.56-1.75 (2H, m), 2.77 (2H, d, J = 5.6 Hz), 4.05-4.20 (1H, m), 4.70 (1H, t, J = 5.6 Hz), 5.08 (2H, s), 7.27-7.36 (5H, m).

Reference example 8

tert-Butoxycarbonyl-L-isoleucyl-L-asparagine

To a stirred solution of tert-butoxycarbonyl-L-isoleucine (2g) in acetonitrile (30ml) was added N-hydroxysuccinimide (1.10g) and dicyclohexylcarbodiimide (1.87g). The reaction mixture was stirred at room temperature for 2 hours, filtered and concentrated. The residue was dissolved in ethanol (20ml). The solution was added to a stirred solution of L-asparagine (1.95g) and sodium hydrogen carbonate (1.1g) in water (30ml). The reaction mixture was stirred at room temperature for 18 hours and was concentrated. The residue was treated with 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to give the title compound (1.1g). ^{1}H-NMR (CD_3OD) δ: 0.80 - 0.96 (6H, m), 1.06-1.30 (1H, m), 1.44 (9H, s), 1.45-1.60 (1H, m), 1.70-1.90 (1H, m), 2.78 (2H, d, J = 5.8 Hz), 3.90-4.00 (1H, m), 4.65-4.80 (1H, m).

Reference example 9

tert-Butoxycarbonyl-L-methionyl-L-asparagine

To a stirred solution of tert-butoxycarbonyl-L-methionine (2g) in acetonitrile (20ml) was added N-hydroxysuccinimide (1.02g) and dicyclohexylcarbodiimide (1.73g). The reaction mixture was stirred

at room temperature for 2 hours, filtered and concentrated. The residue was dissolved in ethanol (20ml). The solution was added to a stirred solution of L-asparagine (1.80g) and sodium hydrogen carbonate (1.0g) in water (20ml). The reaction mixture was stirred at room temperature for 18 hours. The reaction was quenched with 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to give the title compound (2.40g). ^{1}H-NMR (CD_3OD) δ: 1.44 (9H, s), 1.75-2.04 (2H, m), 2.08 (3H, s), 2.47-2.63 (2H, m), 2.77-2.81 (2H, m), 4.10-4.25 (1H, m), 4.65-4.75 (1H, m).

Reference example 10

(S)-3-[(2S,3R,4R,5R)-5-((S)-2-Aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid hydrochloride

To a stirred solution of (S)-2-(tert-butoxycarbonylamino)butyric acid (200mg) and (S)-3-[(2S,3R,4R,5R)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (540mg) in dimethylformamide (10ml) was added diethylphosphorocyanidate (240mg) and triethylamine (0.14ml). The reaction mixture was stirred at room temperature for 17 hours and concentrated. The residue was treated with 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was treated with 4N hydrochloride in ethyl acetate (10ml) at 0° C for 30 min and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (100mg). ^{1}H-NMR (CD_3OD) δ: 1.05 (3H, t, J = 7.6 Hz), 1.85-1.96 (2H, m), 2.74 (2H, d, J = 6.4 Hz), 3.68-4.33 (7H, m), 5.32 (1H, t, J = 6.4 Hz), 7.24-7.42 (5H, m).

Reference example 11

(S)-3-[(2S,3R,4R,5S)-5-((S)-2-Aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-

phenylpropionic acid (2.00g) in methanol (30ml) was added diphenyldiazomethane (1.67g). The reaction mixture was stirred at room temperature for 1.5 hours and concentrated. The residue was washed with ethyl acetate to give the title compound (2.18g). ^{1}H-NMR (CD_3OD) δ : 1.05 (3H, t, J = 7.6 Hz), 1.80-2.00 (2H, m), 3.02-3.15 (2H, m), 3.65-3.73 (3H, m), 3.84-3.90 (2H, m), 4.25-4.33 (2H, m), 5.37-5.48 (1H, m), 6.72 (1H, s), 7.14-7.30 (15H, m).

Reference example 12

(S)-3-[(2S,3R,4R,5S)-5-[N$^{\alpha}$-(9-Fluorenylmethoxycarbonyl)-N$^{\beta}$-triphenylmethyl-L-asparaginyl]amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-N$^{\beta}$-triphenylmethyl-L-asparagine (1.5g) and (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (1.37g) in dimethylformamide (20ml) was added diethyl phosphrocyanidate (614mg) and diisopropylethylamine (0.66ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized (diisopropyl ether) to give the title compound (2.37g). ^{1}H-NMR (CD_3OD) δ : 2.65-2.80 (2H, m), 2.90-3.10 (2H, m), 3.60-3.75 (3H, m), 3.85-3.95 (1H, m), 4.15-4.60 (6H, m), 5.42 (1H, t, J = 6.2 Hz), 6.71 (1H, s), 7.05-7.80 (38H, m).

Reference example 13

(S)-3-[(2S,3R,4R,5S)-5-(N$^{\beta}$-Triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-[N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-N$^{\beta}$-triphenylmethyl-L-asparaginyl]amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-

phenylpropionic acid diphenylmethyl ester (2.03g) in dimethylformamide (10ml) was added piperidine (3ml) at 0° C. The reaction mixture was stirred at room temperature for 2 hours and concentrated. The residue was purified by silica gel column chromatography (eluted with a solution of methanol : ethyl acetate = 1 : 4) and crystallized (diisopropyl ether) to give the title compound (1.30g). ^{1}H-NMR (CD_3OD) δ : 2.50-2.90 (2H, m), 2.95-3.20 (2H, m), 3.60-3.80 (3H, m), 3.85-3.95 (1H, m), 4.04-4.35 (3H, m), 5.35-5.50 (1H, m), 6.72 (1H, s), 7.10-7.40 (30H, m).

Reference example 14

tert-Butoxycarbonyl-L-allylglycyl-L-asparagine

To a stirred solution of tert-butoxycarbonyl-L-allylglycine (800mg) in acetonitrile (20ml) was added N-hydroxysuccinimide (470mg) and dicyclohexylcarbodiimide (806mg). The reaction mixture was stirred at room temperature for 2 hours, filtered and concentrated. The residue was dissolved in ethanol (10ml). The above solution was added to a solution of L-asparagine (888mg) and sodium hydrogen carbonate (468mg) in water (10ml). The reaction mixture was stirred at room temperature for 5 hours. The reaction was quenched with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized (ethyl acetate-diisopropyl ether) to give the title compound (717mg). ^{1}H-NMR (CD_3OD) δ : 1.43 (9H, s), 2.25-2.60 (2H, m), 2.77 (2H, d, J = 5.4 Hz), 4.00-4.20 (1H, m), 4.62-4.74 (1H, m), 5.00-5.20 (2H, m), 5.66-5.90 (1H, m).

Reference example 15

Benzyloxycabonyl-L-norvalyl-(S)-2-aminobutyric acid

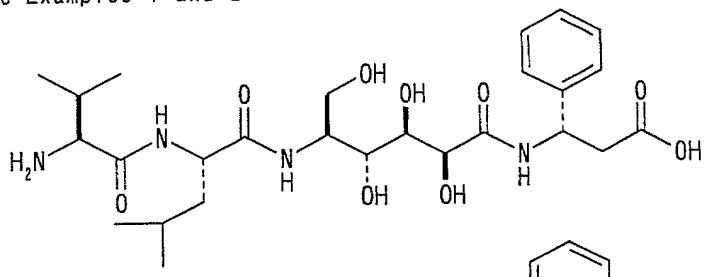
To a stirred solution of benzyloxycabonyl-L-norvaline (30g) in tetrahydrofuran (200ml) was added N-hydroxysuccinimide (15.1g) and dicyclohexylcarbodiimide (25.2g) at 0° C. The reaction mixture was stirred at 0° C for 1 hours and at room temperature for 3 hours, filtered and concentrated. The residue was dissolved in ethanol (200ml). The above solution was added to a solution of (S)-2-aminobutyric acid (15.4g) and sodium hydrogen carbonate (12.5g) in

water (200ml) at 0°C. The reaction mixture was stirred at room temperature for 8 hours. The reaction was quenched with 1N hydrochloric acid (150ml). The precipitate was collected and washed with water to give the title compound (33.5g). ^{1}H-NMR (CD_3OD) δ: 0.89-0.97 (6H, m), 1.32-1.93 (6H, m), 4.13 (1H, m), 4.23-4.25 (1H, m), 5.08 (2H, s), 7.20 (1H, d, J = 8.6 Hz), 7.25-7.33 (5H, m), 8.16 (1H, d, J = 8.4 Hz).

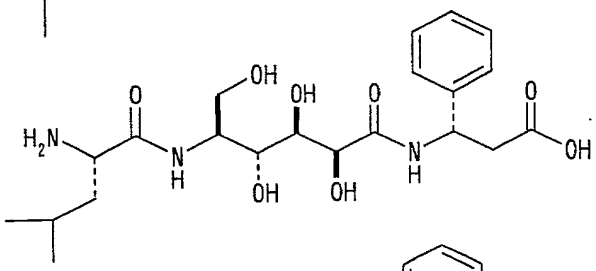
The chemical formulas of the compound obtained above are as follows.

Compounds of Reference Examples 1 and 2

HC-7011



HC-70111



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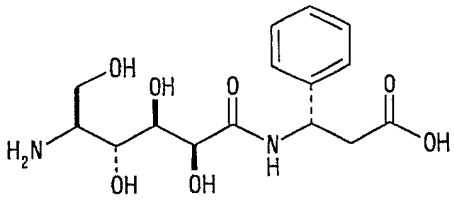

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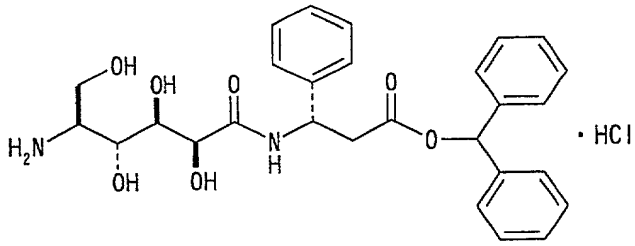

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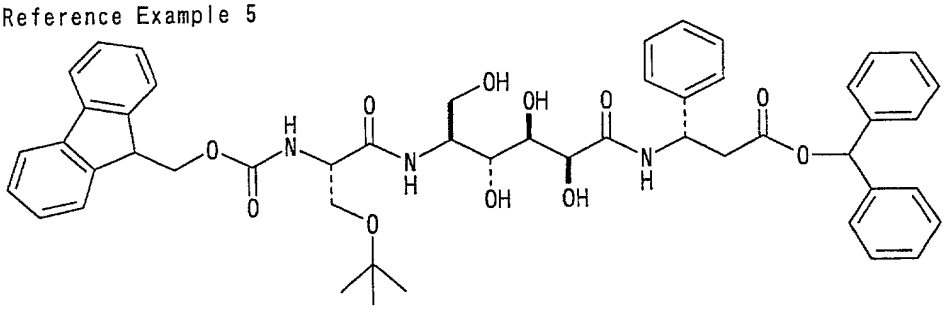

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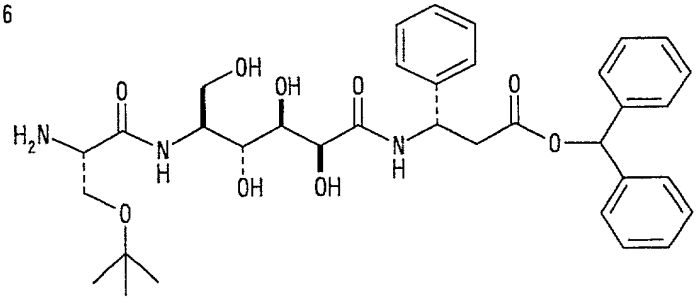

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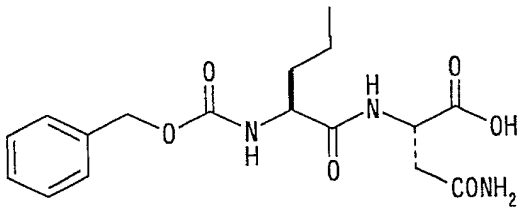
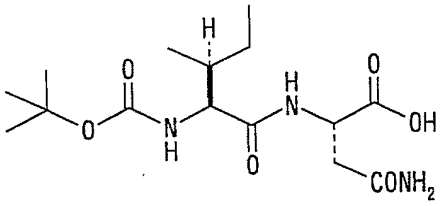


Compound of reference Example 8



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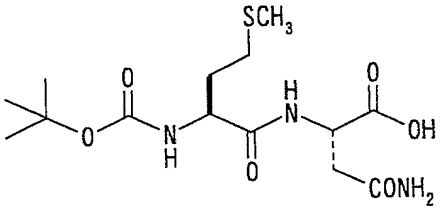

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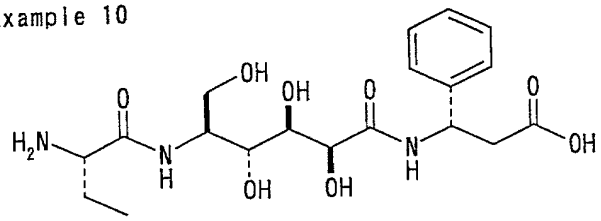
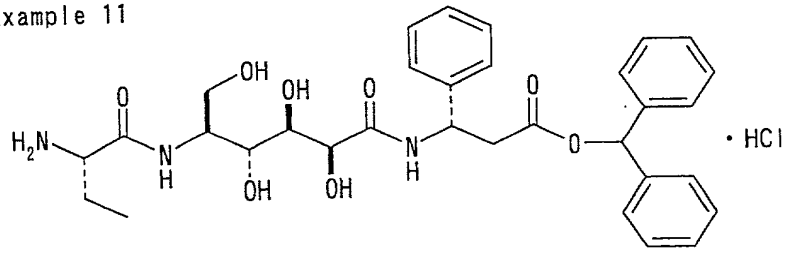


Compound of Reference Example 11



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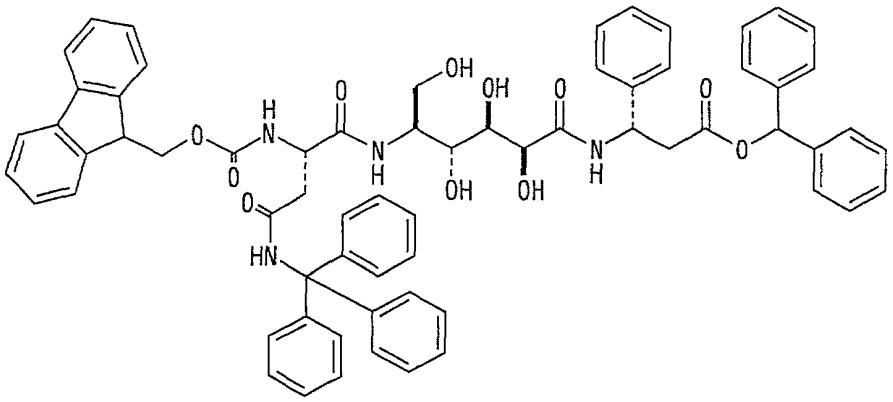

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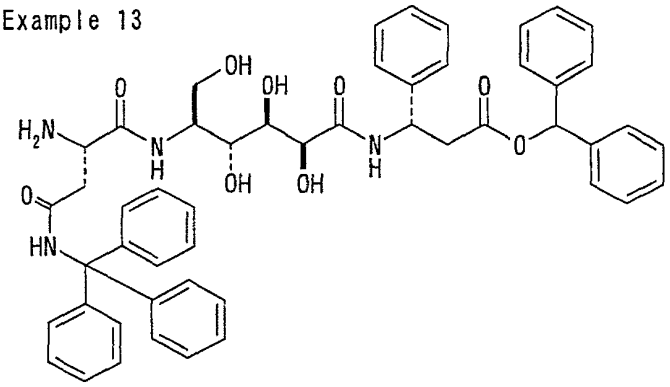

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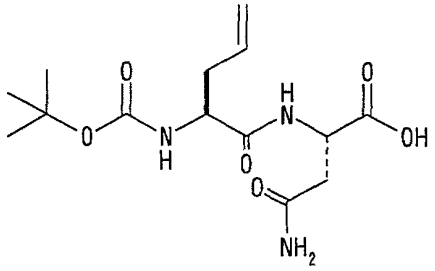

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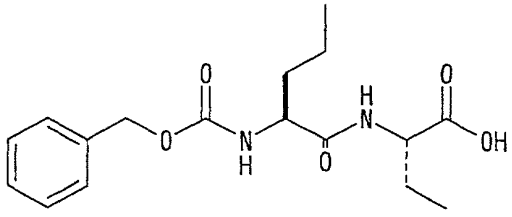

Example 1

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-norvalyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-norvaline (217mg) in acetonitrile (10ml) was added N-hydroxysuccinimide (115mg) and dicyclohexylcarbodiimide (206mg). The reaction mixture was stirred at room temperature for 3 hours, filtered and concentrated. The residue was dissolved in dimethylformamide (5ml). To the above solution was added (S)-3-[(2S,3R,4R,5S)-5-(O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (652mg). The reaction mixture was stirred at room temperature for 18 hours. The reaction was quenched with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized (ether-hexane) to give the title compound (784mg). ^{1}H-NMR (DMSO-d_6) δ: 0.75-1.70 (7H, m), 1.11 (9H, s), 1.38 (9H, s), 3.05 (2H, d, J = 7.0 Hz), 3.20-5.40 (11H, m), 6.67 (1H, s), 7.10-7.60 (15H, m).

Example 2

(S)-3-[(2S,3R,4R,5S)-5-(L-Norvalyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-norvalyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (700mg) was added 4N hydrochloric acid in ethyl acetate (10ml). The reaction mixture was stirred at room temperature for 1 hour and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 20% acetonitrile in water) and recrystallized (methanol-ether) to give the title compound (184mg). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J = 7.4 Hz), 1.20-1.65 (4H, m), 2.50-2.80 (2H, m), 3.00-5.30 (11H, m), 7.10-7.45 (5H, m).

Example 3

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-isoleucyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of N-tert-butoxycarbonyl-L-isoleucine (231mg) in acetonitrile (10ml) was added N-hydroxysuccinimide (115mg) and dicyclohexylcarbodiimide (206mg). The reaction mixture

was stirred at room temperature for 3 hours, filtered and concentrated. The residue was dissolved in dimethylformamide (10ml). To the above solution was added (S)-3-[(2S,3R,4R,5S)-5-(O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (652mg). The reaction mixture was stirred at room temperature for 18 hours. The reaction was quenched with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized (ether-hexane) to give the title compound (647mg). ^{1}H-NMR (DMSO-d_6) δ : 0.70-0.90 (6H, m), 1.00-1.80 (3H, m), 1.10 (9H, s), 1.38 (9H, s), 3.04 (2H, d, J = 7.4 Hz), 3.20-5.40 (11H, m), 6.67 (1H, s), 7.10-7.40 (15H, m).

Example 4

(S)-3-[(2S,3R,4R,5S)-5-(L-Isoleucyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-isoleucyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (550mg) and 4N hydrochloric acid in ethyl acetate (10ml) was stirred at room temperature for 1 hour and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 20% acetonitrile in water) and recrystallized (methanol-ether) to give the title compound (208mg). ^{1}H-NMR (DMSO-d_6) δ : 0.75-0.95 (6H, m), 0.95-1.85 (3H, m), 2.55-2.90 (2H, m), 3.10-5.40 (11H, m), 7.10-7.50 (5H, m).

Example 5

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-methionyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of N-tert-butoxycarbonyl-L-methionine (249mg) in acetonitrile (10ml) was added N-hydroxysuccinimide (115mg) and dicyclohexylcarbodiimide (206mg). The reaction mixture was stirred at room temperature for 3 hours, filtered and concentrated. The residue was dissolved in dimethylformamide (5ml). To the above solution was added (S)-3-[(2S,3R,4R,5S)-5-(O-tert-

butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (652mg). The reaction mixture was stirred at room temperature for 18 hours. The reaction was quenched with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized (ether-hexane) to give the title compound (859mg). ^{1}H-NMR (DMSO-d_6) δ : 1.11 (9H, s), 1.38 (9H, s), 1.60-2.00 (2H, m), 2.01 (3H, s), 2.40-2.60 (2H, m), 3.05 (2H, d, J = 7.2 Hz), 3.20-5.40 (11H, m), 6.67 (1H, s), 7.10-7.40 (15H, m).

Example 6

(S)-3-[(2S,3R,4R,5S)-5-(L-Methionyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-methionyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (750mg) and 4N hydrochloric acid in ethyl acetate (10ml) was stirred at room temperature for 1 hour and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 20% acetonitrile in water) and recrystallized (methanol-ether) to give the title compound (225mg). ^{1}H-NMR (DMSO-d_6) δ : 1.60-2.10 (4H, m), 2.03 (3H, s), 2.60-2.90 (2H, m), 3.00-5.30 (11H, m), 7.10-7.50 (5H, m).

Example 7

(S)-3-[(2S,3R,4R,5S)-5-(Benzyloxycarbonyl-L-norvalyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To a stirred solution of benzyloxycarbonyl-L-norvalyl-L-asparagine (1.0g) in acetonitrile (10ml) was added N-hydroxysuccinimide (346mg) and dicyclohexylcarbodiimide (591mg). The reaction mixture was stirred at room temperature for 5 hours, filtered and concentrated. The residue was dissolved in ethanol (50ml). To the above solution was added a solution of (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid (1.40g) and sodium hydrogen carbonate (344mg)

in water (10ml). The reaction mixture was stirred at room temperature for 18 hours. The reaction was quenched with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (diisopropyl ether) to give the title compound (1.08g). ^{1}H-NMR (CD_3OD) δ: 0.92 (3H, t, J = 6.8 Hz), 1.37-1.44 (2H, m), 1.64-1.71 (2H, m), 2.65-2.76 (2H, m), 2.84-2.91 (2H, m), 3.64-3.77 (3H, m), 3.90 (1H, dd, J = 1.0, 10.2 Hz), 4.00-4.25 (2H, m), 4.25-4.28 (1H, m), 4.65-4.80 (1H, m), 5.10 (2H, s), 5.30-5.45 (1H, m), 7.23-7.40 (10H, m).

Example 8

(S)-3-[(2S,3R,4R,5S)-5-(L-Norvalyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A solution of (S)-3-[(2S,3R,4R,5S)-5-(benzyloxycarbonyl-L-norvalyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid (300mg) in methanol (20ml) was stirred with 10% palladium on charcoal (50mg) under a hydrogen atmosphere at room temperature for 2 hours, filtered and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (45mg). ^{1}H-NMR (D_2O) δ: 0.89 (3H, t, J = 7.4 Hz), 1.28-1.40 (2H, m), 1.50-1.84 (2H, m), 2.69-2.78 (4H, m), 3.61-3.74 (4H, m), 3.85-3.98 (2H, m), 4.24 (1H, t, J = 6.0 Hz), 4.33 (1H, s), 5.18 (1H, t, J = 6.8 Hz), 7.30-7.38 (5H, m).

Example 9

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-isoleucyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-isoleucyl-L-asparagine (500mg) in acetonitrile (20ml) was added N-hydroxysuccinimide (183mg) and dicyclohexylcarbodiimide (314mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl

ester hydrochloride (790mg) and diisopropylethylamine (0.51ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (diisopropyl ether) to give the title compound (1.00g). ^{1}H-NMR (CD_3OD) δ : 0.88-0.93 (6H, m), 1.10-1.30 (1H, m), 1.44 (9H, s), 1.45-1.55 (1H, m), 1.75-1.85 (1H, m), 2.68-2.78 (2H, m), 3.00-3.19 (2H, m), 3.60-3.77 (3H, m), 3.88-4.00 (2H, m), 4.15-4.20 (1H, m), 4.22-4.25 (1H, m), 4.70-4.90 (1H, m), 5.44 (1H, t, J = 7.0 Hz), 6.74 (1H, s), 7.16-7.32 (15H, m).

Example 10

(S)-3-[(2S,3R,4R,5S)-5-(L-Isoleucyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-isoleucyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxy-hexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (400mg) and trifluoroacetic acid (20ml) was stirred at room temperature for 30 min and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (140mg). ^{1}H-NMR (D_2O) δ : 0.85-0.98 (6H, m), 1.09-1.22 (2H, m), 1.35-1.60 (1H, m), 2.65-2.88 (4H, m), 3.50-3.71 (4H, m), 3.84-3.89 (2H, m), 4.23 (1H, t, J = 8.0Hz), 4.30-4.36 (1H, m), 5.20 (1H, t, J = 7.0Hz), 7.25-7.35 (5H, m).

Example 11

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-methionyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-methionyl-L-asparagine (500mg) and (S)-3-((2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl)amino-3-phenylpropionic acid diphenylmethyl

ester hydrochloride (425mg) in dimethylformamide (10ml) was added diethyl phosphorocyanidate (191mg) and diisopropylethylamine (0.2ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (diisopropyl ether) to give the title compound (340mg). ^{1}H-NMR (CD_3OD) δ: 1.43 (9H, s), 1.85-2.00 (2H, m), 2.05 (3H, s), 2.47-2.57 (2H, m), 3.00-3.13 (4H, m), 3.64-4.20 (6H, m), 4.30-4.34 (1H, d, J = 1.4 Hz), 4.60-4.80 (1H, m), 5.44 (1H, t, J = 7.6 Hz), 6.73 (1H, s), 7.14-7.31 (15H, m).

Example 12

(S)-3-[(2S,3R,4R,5S)-5-(L-Methionyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-methionyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (200mg) and trifluoroacetic acid (5ml) was stirred at room temperature for 15 min and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (30mg). ^{1}H-NMR (D_2O) δ: 2.06 (3H, s), 2.15 (2H, t, J = 7.2 Hz), 2.57 (2H, t, J = 7.2 Hz), 2.68-2.80 (4H, m), 3.56-3.74 (4H, m), 3.83-3.88 (1H, m), 4.02-4.14 (1H, m), 4.23 (1H, t, J = 6.2Hz), 4.30-4.35 (1H, m), 5.16 (1H, t, J = 7.0 Hz), 7.25-7.35 (5H, m).

Example 13

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution L-norvaline (115mg) in tetrahydrofuran (10ml) and water (10ml) was added di-tert-butyl dicarbonate (0.248ml) and sodium hydrogen carbonate (247mg). The reaction mixture was stirred at room temperature for 2 hours. The reaction was quenched with 5% citric acid and extracted with ethyl acetate.

The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was dissolved in acetonitrile (10ml). To the above solution was added N-hydroxysuccinimide (134mg) and dicyclohexylcarbodiimide (220mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. To the filtrate was added a solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (600mg) and diisopropylethylamine (0.34ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-hexane) to give the title compound (755mg). ^{1}H-NMR (CD_3OD) δ: 0.80-0.96 (6H, m), 1.20-1.30 (2H, m), 1.43 (9H, s), 1.58-1.88 (4H, m), 3.05 (1H, dd, J = 15.6, 7.0 Hz), 3.10 (1H, dd, J = 7.0, 15.6 Hz), 3.63-3.71 (3H, m), 3.88-4.04 (2H, m), 4.16-4.31 (3H, m), 5.43 (1H, t, J = 7.0 Hz), 6.73 (1H, s), 7.15-7.30 (15H, m).

Example 14

(S)-3-[(2S,3R,4R,5S)-5-(L-Norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (500mg) in ethyl acetate (10ml) was added 4N hydrochloric acid in ethyl acetate (30ml). The reaction mixture was stirred at room temperature for 2 hours and concentrated. The residue was washed with ether and purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (70mg). ^{1}H-NMR (D_2O) δ: 0.80-1.00 (6H, m), 1.20-1.45 (2H, m), 1.55-1.90 (4H, m), 2.68 (2H, d, J = 7.0 Hz), 3.52-3.74 (3H, m), 3.81-4.00 (2H, m), 4.16-4.33 (3H, m), 5.14 (1H, t, J = 7.0 Hz), 7.20-7.40 (5H, m).

Example 15

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-isoleucyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-isoleucine (224mg) in acetonitrile (10ml) was added N-hydroxysuccinimide (134mg) and dicyclohexylcarbodiimide (220mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (600mg) and diisopropylethylamine (0.34ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 19 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-hexane) to give the title compound (658mg). ^{1}H-NMR (CD_3OD) δ: 0.80-0.96 (9H, m), 1.10-1.16 (1H, m), 1.43 (9H, s), 1.45-1.55 (1H, m), 1.60-1.90 (3H, m), 3.02 (1H, dd, J = 7.8, 15.6 Hz), 3.12 (1H, dd, J = 5.6, 15.6 Hz), 3.59-3.73 (3H, m), 3.89-3.93 (2H, m), 4.16-4.24 (1H, m), 4.28-4.34 (2H, m), 5.43 (1H, dd, J = 5.6, 7.8 Hz), 6.72 (1H, s), 7.15-7.30 (15H, m).

Example 16

(S)-3-[(2S,3R,4R,5S)-5-(L-Isoleucyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-isoleucyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (300mg) in ethyl acetate (10ml) was added 4N hydrochloric acid in ethyl acetate (20ml). The reaction mixture was stirred at room temperature for 2 hours and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (80mg). ^{1}H-NMR (D_2O) δ: 0.89-0.95 (9H, m), 1.15-1.30 (1H, m), 1.40-1.60 (1H, m), 1.71-2.04 (3H, m), 2.75 (2H, d, J = 7.2 Hz), 3.63-3.73 (3H, m), 3.86-3.91 (2H, m), 4.21-4.34

(3H, m), 5.19 (1H, t, J = 7.2 Hz), 7.25-7.35 (5H, m).

Example 17

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-methionyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-methione (242mg) in acetonitrile (10ml) was added N-hydroxysuccinimide (134mg) and dicyclohexylcarbodiimide (220mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (600mg) and diisopropylethylamine (0.34ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 19 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-hexane) to give the title compound (663mg). ^{1}H-NMR (CD_3OD) δ : 0.97 (3H, t, J = 7.2 Hz), 1.43 (9H, s), 1.60-1.77 (2H, m), 1.81-1.92 (2H, m), 2.06 (3H, s), 2.48-2.60 (2H, m), 3.02 (1H, dd, J = 7.6, 15.6 Hz), 3.13 (1H, dd, J = 6.2, 15.6 Hz), 3.59-3.71 (3H, m), 3.87-3.93 (1H, m), 4.15-4.32 (4H, m), 5.43 (1H, dd, J = 6.2, 7.6 Hz), 6.73 (1H, s), 7.14-7.32 (15H, m).

Example 18

(S)-3-[(2S,3R,4R,5S)-5-(L-Methionyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-methionyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (300mg) in ethyl acetate (10ml) was added 4N hydrochloric acid in ethyl acetate (20ml) was stirred at room temperature for 2 hours and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (70mg). ^{1}H-NMR (D_2O) δ : 0.92 (3H, t, J = 7.6 Hz), 1.72-1.87

(2H, m), 2.07 (3H, s), 2.10-2.21 (2H, m), 2.58 (2H, t, J = 7.4 Hz), 2.70 (2H, d, J = 6.8 Hz), 3.63-3.72 (3H, m), 3.84-3.90 (1H, m), 4.12 (1H, t, J = 7.0 Hz), 4.20-4.35 (3H, m), 5.16 (1H, t, J = 6.8 Hz), 7.25-7.36 (5H, m).

Example 19

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-asparaginyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-asparagine (196mg) in acetonitrile (10ml) was added N-hydroxysuccinimide (116mg) and dicyclohexylcarbodiimide (183mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-(O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (500mg) and diisopropylethylamine (0.13ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-diisopropyl ether) to give the title compound (570mg). ^{1}H-NMR (CD_3OD) δ: 1.20 (9H, s), 1.43 (9H, s), 2.60 (1H, dd, J = 6.8, 15.2 Hz), 2.78 (1H, dd, J = 6.8, 15.2 Hz), 3.01 (1H, dd, J = 8.0, 15.8 Hz), 3.13 (1H, dd, J = 6.0, 15.8 Hz), 3.55-3.84 (5H, m), 3.88-3.98 (1H, m), 4.20-4.55 (4H, m), 5.43 (1H, dd, J = 6.0, 8.0 Hz), 6.73 (1H, s), 7.10-7.40 (15H, m).

Example 20

(S)-3-[(2S,3R,4R,5S)-5-(L-Asparaginyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-asparaginyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (400mg) and trifluoroacetic acid (10ml) was stirred at room temperature for 30 min and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10%

acetonitrile in water) and recrystallized (methanol-ether) to give the title compound (150mg). ^{1}H-NMR (D_2O) δ: 2.80-3.00 (4H, m), 3.55-3.75 (3H, m), 3.80-3.95 (3H, m), 4.20-4.45 (3H, m), 4.50-4.60 (1H, m), 5.20-5.40 (1H, m), 7.30-7.50 (5H, m).

Example 21

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-allylglycyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-allylglycine (198mg), (S)-3-[(2S,3R,4R,5S)-5-(O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (600mg) and N-hydroxysuccinimide (212mg) in dimethylformamide (10ml) was added dicyclohexylcarbodiimide (237mg). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified with silica gel column chromatography (eluted with ethyl acetate) and crystallized (ethyl acetate-diisopropyl ether) to give the title compound (475mg). ^{1}H-NMR (CD_3OD) δ: 1.21 (9H, s), 1.44 (9H, s), 2.30-2.60 (2H, m), 2.95-3.20 (2H, m), 3.55-3.80 (5H, m), 3.88-4.00 (1H, m), 4.05-4.50 (4H, m), 5.00-5.20 (2H, m), 5.40-5.50 (1H, m), 5.70-5.90 (1H, m), 6.73 (1H, s), 7.10-7.40 (15H, m).

Example 22

(S)-3-[(2S,3R,4R,5S)-5-(L-Allylglycyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-allylglycyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (350mg) and trifluoroacetic acid (5ml) was stirred at room temperature for 30 min and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 20% acetonitrile in water) and recrystallized (methanol) to give the title compound (163mg). ^{1}H-NMR (D_2O) δ: 2.55-2.75 (4H, m),

3.60-3.72 (3H, m), 3.78-3.92 (3H, m), 4.04-4.16 (1H, m), 4.18-4.36 (2H, m), 4.44-4.60 (1H, m), 5.10-5.30 (3H, m), 5.60-5.85 (1H, m), 7.20-7.45 (5H, m).

Example 23

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-S-methyl-L-cysteinyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of in tert-butoxycarbonyl-L-cysteine (199mg) in acetonitrile (10ml) were added N-hydroxysuccinimide (116mg) and dicyclohexylcarbodiimide (183mg). The reaction mixture was stirred at room temperature for 2 hours, filtered. The filtrate was added to a solution of (S)-3-[(2S,3R,4R,5S)-5-(O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (500mg) and diisopropylethylamine (0.13ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified with silica gel column chromatography (eluted with ethyl acetate) and crystallized (ethyl acetate-diisopropyl ether) to give the title compound (560mg). ^{1}H-NMR (CD_3OD) δ : 1.20 (9H, s), 1.44 (9H, s), 2.11 (3H, s), 2.60-3.20 (4H, m), 3.55-3.80 (5H, m), 3.88-3.98 (1H, m), 4.16-4.46 (4H, m), 5.43 (1H, t, J = 6.0 Hz), 6.73 (1H, s), 7.10-7.40 (15H, m).

Example 24

(S)-3-[(2S,3R,4R,5S)-5-(S-Methyl-L-cysteinyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-S-methyl-L-cysteinyl-O-tert-butyl-L-seryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (400mg) and trifluoroacetic acid (10ml) was stirred at room temperature for 30 min and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ether) to give

the title compound (114mg). ^{1}H-NMR (D_2O) δ: 2.10 (3H, s), 2.85-3.20 (4H, m), 3.36-3.75 (3H, m), 3.80-3.90 (3H, m), 4.15-4.35 (3H, m), 4.48-4.60 (1H, m), 5.20-5.40 (1H, m), 7.20-7.45 (5H, m).

Example 25

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-asparaginyl-N$^{\beta}$-triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-asparagine (168mg) and (S)-3-[(2S,3R,4R,5S)-5-(N$^{\beta}$-triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (500mg) in dimethylformamide (10ml) were added N-hydroxysuccinimide (133mg) and dicyclohexylcarbodiimide (164mg) at 0° C. The reaction mixture was stirred at room temperature for 18 hours, filtered and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified with silica gel column chromatography (eluted with ethyl acetate : methanol = 10 : 1) and crystallized (methanol-diisopropyl ether) to give the title compound (521mg). ^{1}H-NMR (CD_3OD) δ: 1.41 (9H, s), 2.60-2.75 (2H, m), 2.90 (2H, d, J = 7.4 Hz), 3.00-3.10 (2H, m), 3.60-3.75 (4H, m), 4.10-4.80 (4H, m), 5.42 (1H, t, J = 7.4 Hz), 6.73 (1H, s), 7.10-7.40 (30H, m).

Example 26

(S)-3-[(2S,3R,4R,5S)-5-(L-Asparaginyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-asparaginyl-N$^{\beta}$-triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (350mg) and trifluoroacetic acid (5ml) was stirred at room temperature for 1 hour and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate)

to give the title compound (90mg). ^{1}H-NMR (D_2O) δ : 2.70-3.00 (6H, m), 3.55-3.75 (4H, m), 3.80-3.90 (1H, m), 4.14-4.34 (3H, m), 5.21 (1H, t, J = 7.0 Hz), 7.20-7.45 (5H, m).

Example 27

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-allylglycyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-allylglycyl-L-asparagine (300mg) and (S)-3-((2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl)amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (496mg) in dimethylformamide (10ml) were added diethyl phosphorocyanidate (223mg) and diisopropylethylamine (0.24ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (diisopropyl ether) to give the title compound (553mg). ^{1}H-NMR (CD_3OD) δ : 1.43 (9H, s), 2.20-2.60 (2H, m), 2.72 (2H, d, J = 6.2 Hz), 2.95-3.20 (2H, m), 3.60-3.78 (3H, m), 3.85-3.95 (1H, m), 4.00-4.35 (4H, m), 5.00-5.20 (2H, m), 5.43 (1H, t, J = 6.2 Hz), 5.65-5.90 (1H, m), 6.73 (1H, s), 7.10-7.40 (15H, m).

Example 28

(S)-3-[(2S,3R,4R,5S)-5-(L-Allylglycyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-allylglycyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (130mg) and trifluoroacetic acid (5ml) was stirred at room temperature for 30 min and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (47mg). ^{1}H-NMR (D_2O) δ : 2.55-2.85 (6H, m), 3.59-3.74 (4H, m), 3.80-3.90 (1H, m), 4.00-4.10 (1H, m),

4.16-4.27 (1H, m), 4.28-4.32 (1H, m), 5.17-5.27 (3H, m), 5.60-5.80 (1H, m), 7.20-7.45 (5H, m).

Example 29

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-S-methyl-L-cysteinyl-N$^{\beta}$-triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-S-methyl-L-cysteine (170mg) and (S)-3-[(2S,3R,4R,5S)-5-(N$^{\beta}$-triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (500mg) in dimethylformamide (10ml) were added N-hydroxysuccinimide (133mg) and dicyclohexylcarbodiimide (164mg) at 0° C. The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine and saturated aqueous sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate and concentrated. The residue was purified with silica gel column chromatography (eluted with a solution of ethyl acetate : methanol = 10 : 1) and crystallized (methanol-diisopropyl ether) to give the title compound (507mg).

^{1}H-NMR (CD_3OD) δ : 1.42 (9H, s), 2.10 (3H, s), 2.60-3.20 (6H, m), 3.60-3.80 (3H, m), 3.85-3.95 (1H, m), 4.10-4.70 (4H, m), 5.43 (1H, t, J = 7.8 Hz), 6.73 (1H, s), 7.05-7.40 (30H, m).

Example 30

(S)-3-[(2S,3R,4R,5S)-5-(S-Methyl-L-cysteinyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-S-methyl-L-cysteinyl-N$^{\beta}$-triphenylmethyl-L-asparaginyl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (300mg) and trifluoroacetic acid (5ml) was stirred at room temperature for 1 hour and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized

(methanol-ether) to give the title compound (120mg). ^{1}H-NMR (D_2O) δ: 2.10 (3H, s), 2.65-3.15 (6H, m), 3.55-3.78 (4H, m), 3.79-3.90 (1H, m), 4.12-4.40 (3H, m), 5.20 (1H, t, J = 6.8 Hz), 7.20-7.50 (5H, m).

Example 31

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-asparaginyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-asparagine (750mg) in acetonitrile (10ml) were added N-hydroxysuccinimide (409mg) and dicyclohexylcarbodiimide (700mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (2.0g) and diisopropylethylamine (1.1ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-hexane) to give the title compound (2.3g). ^{1}H-NMR (CD_3OD) δ: 0.98 (3H, t, J = 7.4 Hz), 1.42 (9H, s), 1.61-1.76 (1H, m), 1.86-1.96 (1H, m), 2.60 (1H, dd, J = 7.4, 14.8 Hz), 2.76 (1H, dd, J = 7.0, 14.8 Hz), 3.02 (1H, dd, J = 7.4, 15.8Hz), 3.12 (1H, dd, J = 7.4, 15.8Hz), 3.65-3.72 (3H, m), 3.88 (1H, dd, J = 1.6, 9.8 Hz), 4.18-4.27 (2H, m), 4.30-4.35 (1H, m), 4.43 (1H, t, J = 7.4 Hz), 5.43 (1H, t, J = 7.4 Hz), 6.73 (1H, s), 7.14-7.33 (15H, m).

Example 32

(S)-3-[(2S,3R,4R,5S)-5-(L-Asparaginyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-asparaginyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (1.2g) in ethyl acetate (10ml) was added 4N hydrochloric acid in ethyl acetate (10ml). The reaction mixture was stirred at room

temperature for 2 hours and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 5% acetonitrile in water) and recrystallized (methanol-ether) to give the title compound (250mg). ^{1}H-NMR (D_2O) δ: 0.95 (3H, t, J = 7.4 Hz), 1.72-1.87 (2H, m), 2.87-3.00 (4H, m), 3.54-3.79 (3H, m), 3.90 (1H, d, J = 9.8 Hz), 4.15-4.41 (4H, m), 5.32 (1H, t, J = 6.6 Hz), 7.30-7.42 (5H, m).

Example 33

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-L-allylglycyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-L-allylglycine (480mg) in acetonitrile (10ml) were added N-hydroxysuccinimide (282mg) and dicyclohexylcarbodiimide (483mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (1.38g) and diisopropylethylamine (0.78ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-diisopropyl ether) to give the title compound (1.53g). ^{1}H-NMR (CD_3OD) δ: 0.97 (3H, t, J = 7.6 Hz), 1.42 (9H, s), 1.60-1.90 (2H, m), 2.25-2.60 (2H, m), 3.02 (1H, dd, J = 6.9, 15.6 Hz), 3.13 (1H, dd, J = 6.9, 15.6 Hz), 3.59-3.74 (3H, m), 3.87-3.93 (1H, m), 4.06-4.43 (4H, m), 5.00-5.20 (2H, m), 5.43 (1H, t, J = 6.9 Hz), 5.64-5.90 (1H, m), 6.73 (1H, s), 7.13-7.30 (15H, m).

Example 34

(S)-3-[(2S,3R,4R,5S)-5-(L-Allylglycyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-L-allylglycyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl

ester (400mg) and trifluoroacetic acid (5ml) was stirred at room temperature for 2 hour and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (210mg). ^{1}H-NMR (D_2O) 0.89 (3H, t, J = 7.2 Hz), 1.60-1.90 (2H, m), 2.50-2.70 (2H, m), 2.80 (2H, d, J = 7.0Hz), 3.55-3.74 (3H, m), 3.80-3.90 (1H, m), 4.00-4.12 (1H, m), 4.14-4.35 (3H, m), 5.10-5.30 (3H, m), 5.55-5.80 (1H, m), 7.20-7.50 (5H, m).

Example 35

(S)-3-[(2S,3R,4R,5S)-5-(tert-Butoxycarbonyl-S-methyl-L-cysteinyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of tert-butoxycarbonyl-S-methyl-L-cysteine (400mg) in acetonitrile (10ml) were added N-hydroxysuccinimide (215mg) and dicyclohexylcarbodiimide (368mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (1.05g) and diisopropylethylamine (0.59ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate-diisopropyl ether) to give the title compound (1.21g). ^{1}H-NMR (CD_3OD) δ: 0.97 (3H, t, J = 7.6 Hz), 1.44 (9H, s), 1.60-2.00 (2H, m), 2.11 (3H, s), 2.70 (1H, dd, J = 8.6, 13.8 Hz), 2.90 (1H, dd, J = 5.6, 13.8 Hz), 3.02 (1H, dd, J = 6.0, 15.8 Hz), 3.12 (1H, dd, J = 6.0, 15.8 Hz), 3.59-3.76 (3H, m), 3.84-3.92 (1H, m), 4.14-4.34 (4H, m), 5.43 (1H, t, J = 6.0 Hz), 6.73 (1H, s), 7.10-7.40 (15H, m).

Example 36

(S)-3-[(2S,3R,4R,5S)-5-(S-Methyl-L-cysteinyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-

phenylpropionic acid

A mixture of (S)-3-[(2S,3R,4R,5S)-5-(tert-butoxycarbonyl-S-methyl-L-cysteinyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (700mg) and trifluoroacetic acid (10ml) was stirred at room temperature for 30 min and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 10% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (100mg). ^{1}H-NMR (D_2O) δ: 0.90 (3H, t, J = 7.6 Hz), 1.64-1.89 (2H, m), 2.09 (3H, s), 2.69 (2H, d, J = 7.0 Hz), 2.90 (1H, dd, J = 8.2, 14.6 Hz), 3.09 (1H, dd, J = 5.6, 14.6 Hz), 3.55-3.90 (4H, m), 4.14-4.31 (4H, m), 5.15 (1H, t, J = 7.0 Hz), 7.20-7.45 (5H, m).

Example 37

(S)-3-[(2S,3R,4R,5S)-5-(N^{α}-Benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyl-L-lysyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyl-L-lysine (308mg) in acetonitrile (10ml) were added N-hydroxysuccinimide (102mg) and dicyclohexylcarbodiimide (175mg). The reaction mixture was stirred at room temperature for 2 hours and filtered. The filtrate was added to a solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (500mg) and diisopropylethylamine (0.28ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (diisopropyl ether) to give the title compound (700mg). ^{1}H-NMR (CD_3OD) δ: 0.96 (3H, t, J = 7.4 Hz), 1.20-2.00 (8H, m), 1.40 (9H, s), 2.90-3.20 (4H, m), 3.55-3.75 (3H, m), 3.85-3.95 (1H, m), 4.00-4.40 (4H, m), 5.07 (2H, s), 5.43 (1H, t, J = 6.4 Hz), 6.72 (1H, s), 7.10-7.40 (20H, m).

Example 38

(S)-3-[(2S,3R,4R,5S)-5-(L-Lysyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A solution of (S)-3-[(2S,3R,4R,5S)-5-(N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyl-L-lysyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (400mg) in methanol (20ml) was stirred with 10% palladium on charcoal (40mg) under a hydrogen atmosphere at room temperature for 1 hour, filtered and concentrated. The residue was treated with 4N hydrochloric acid in ethyl acetate (10ml) at room temperature for 1 hour, filtered and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 5% acetonitrile in water) and recrystallized (methanol-ethyl acetate) to give the title compound (100mg). ^{1}H-NMR (D_2O) δ: 0.80-1.00 (3H, m), 1.30-2.00 (8H, m), 2.80-3.10 (4H, m), 3.60-4.50 (8H, m), 5.20-5.40 (1H, m), 7.30-7.50 (5H, m).

Example 39

(S)-3-[(2S,3R,4R,5S)-5-(Benzyloxycarbonyl-L-valyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester

To a stirred solution of benzyloxycarbonyl-L-valine (400mg) in acetonitrile (10ml) were added N-hydroxysuccinimide (202mg) and dicyclohexylcarbodiimide (344mg). The reaction mixture was stirred at room temperature for 3 hours and filtered. The filtrate was added to a solution of (S)-3-[(2S,3R,4R,5S)-5-((S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester hydrochloride (983mg) and diisopropylethylamine (0.55ml) in dimethylformamide (10ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with 5% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (diisopropyl ether) to give the title compound (1.12g). ^{1}H-NMR (CD_3OD) δ: 0.80-1.00 (9H, m), 1.50-2.00 (3H, m), 2.90-3.20 (2H, m), 3.60-3.80 (3H, m), 3.85-4.00 (2H, m),

4.15-4.40 (3H, m), 5.09 (2H, s), 5.44 (1H, t, J = 7.0 Hz), 6.73 (1H, s), 7.10-7.40 (20H, m).

Example 40

(S)-3-[(2S,3R,4R,5S)-5-(L-Valyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

A solution of (S)-3-[(2S,3R,4R,5S)-5-(benzyloxycarbonyl-L-valyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid diphenylmethyl ester (500mg) in methanol (30ml) was stirred with 10% palladium on charcoal (50mg) under a hydrogen atmosphere at room temperature for 3 hours, filtered and concentrated. The residue was purified by DIAION CHP-20P column chromatography (eluted with water and 5% acetonitrile in water) and recrystallized (ethanol-ethyl acetate) to give the title compound (209mg). ^{1}H-NMR (D_2O) δ : 0.91-1.00 (9H, m), 1.67-1.85 (2H, m), 2.16 (1H, m), 2.69 (2H, d, J = 6.6 Hz), 3.62-3.78 (4H, m), 3.87 (1H, d, J = 9.8 Hz), 4.21-4.33 (3H, m), 5.15 (1H, t, J = 6.6 Hz), 7.20-7.35 (5H, m).

Example 41

(S)-3-[(2S,3R,4R,5S)-5-(Benzyloxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid

To a stirred solution of benzyloxycarbonyl-L-norvalyl-(S)-2-aminobutyric acid (30g), N-hydroxysuccinimide (11.3g) in tetrahydrofuran (300ml) was added dicyclohexylcarbodiimide (19.3g) at 0° C. The reaction mixture was stirred at 0° C for 1 hour and at room temperature for 1 hour, filtered and concentrated. The residue was dissolved in dimethylformamide (150ml). The above solution was added to a solution of (S)-3-[(2S,3R,4R,5S)-5-amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid (39.7g) and triethylamine (16.2ml) in dimethylformamide (200ml). The reaction mixture was stirred at room temperature for 18 hours and concentrated. The residue was treated with hydrochloric acid and extracted with a solution of ethyl acetate and tetrahydrofuran. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was crystallized (ethyl acetate) to give the title compound (45.5g). ^{1}H-NMR (CD_3OD) δ :

0.80-1.05 (6H, m), 1.10-1.90 (6H, m), 2.70-3.00 (2H, m), 3.60-3.80 (3H, m), 3.85-3.95 (1H, m), 4.00-4.40 (4H, m), 5.09 (2H, s), 5.30-5.50 (1H, m), 7.20-7.45 (10H, m).

Example 42

(S)-3-[(2S,3R,4R,5S)-5-(L-Norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid (another synthetic method of the compound of example 14)

A solution of (S)-3-[(2S,3R,4R,5S)-5-(benzyloxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid (19.5g) in methanol (300ml) and water (30ml) was stirred with 10% palladium on charcoal (1.9g) under a hydrogen atmosphere at room temperature for 5 hours, filtered and concentrated. The residue recrystallized (ethanol-water) to give the title compound (11.5g).

Example 43

(S)-3-[(2S,3R,4R,5S)-5-(Benzyloxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid pivaloyloxymethyl ester

To a stirred solution of (S)-3-[(2S,3R,4R,5S)-5-(benzyloxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid (3.0g) in methanol (30ml) was added a solution of cesium carbonate (740mg) in water (0.5ml). The mixture was stirred at room temperature for 30 min and concentrated. To the residue were added dimethylformamide (30ml) and iodomethyl pivalate (1.10g). The mixture was stirred at room temperature for 2 days and concentrated. The residue was treated with water and extracted with ethyl acetate. The organic layer was washed with brine and saturated aqueous sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate and concentrated. The residue was purified with silica gel column chromatography (eluted with a solution of ethyl acetate : methanol = 10 : 1) and recrystallized (methanol-diisopropyl ether) to give the title compound (2.53g). ^{1}H-NMR (CD_3OD) δ : 0.80-1.05 (6H, m), 1.26 (9H, s), 1.30-1.90 (6H, m), 2.85-3.10 (2H, m), 3.60-3.80 (4H, m), 3.85-3.95 (1H, m), 4.10-4.35 (3H, m), 5.09 (2H, s), 5.41 (1H, t, J = 6.6 Hz), 5.66 (2H, s), 7.20-7.45 (10H, m).

Example 44

(S)-3-[(2S,3R,4R,5S)-5-(L-Norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid pivaloyloxymethyl ester

A solution of (S)-3-[(2S,3R,4R,5S)-5-(benzyloxycarbonyl-L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid pivaloyloxymethyl ester (2.00g) in methanol (30ml) was stirred with 10% palladium on charcoal (150mg) under a hydrogen atmosphere at room temperature for 5 hours, filtered and concentrated. The residue recrystallized (ethanol-diisopropyl ether) to give the title compound (810mg). ^{1}H-NMR (CD_3OD) δ: 0.80-1.05 (6H, m), 1.11 (9H, s), 1.30-1.95 (6H, m), 2.90-3.15 (2H, m), 3.55-3.75 (4H, m), 3.85-4.00 (1H, m), 4.10-4.40 (3H, m), 5.42 (1H, t, J = 7.0 Hz), 5.66 (2H, s), 7.20-7.45 (5H, m).

The chemical formulas of the compounds obtained in the above Examples are as follows.

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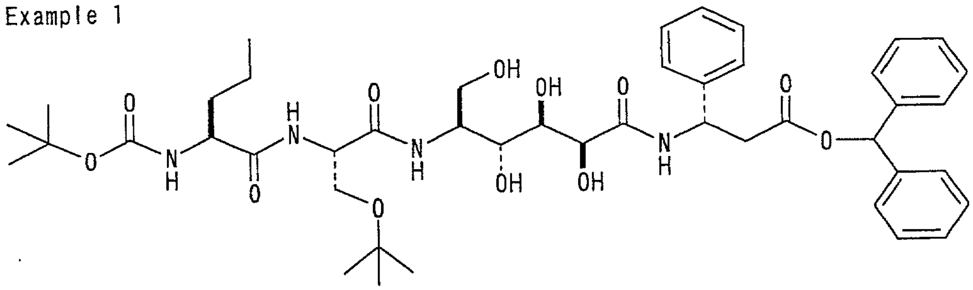

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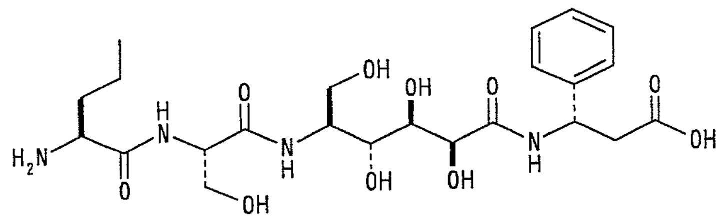

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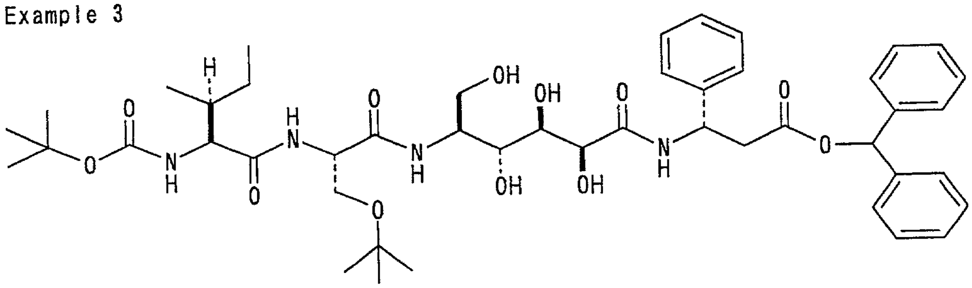

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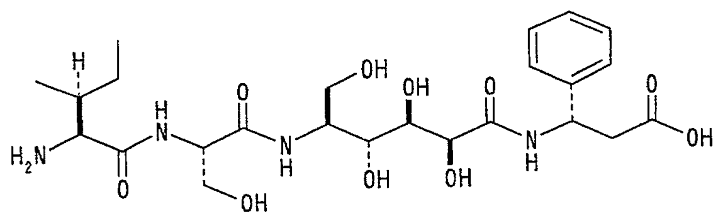

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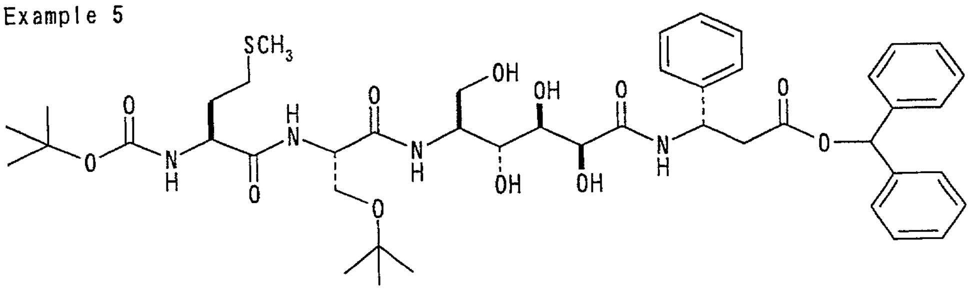

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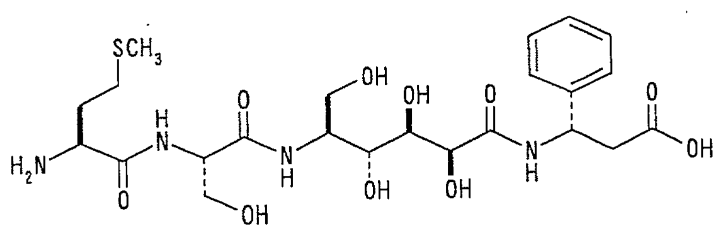

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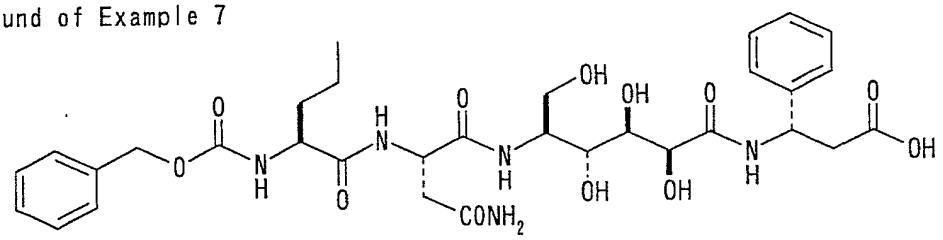

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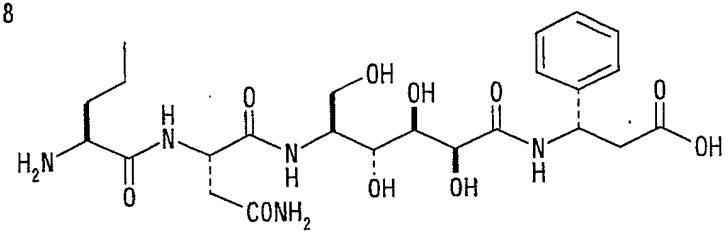

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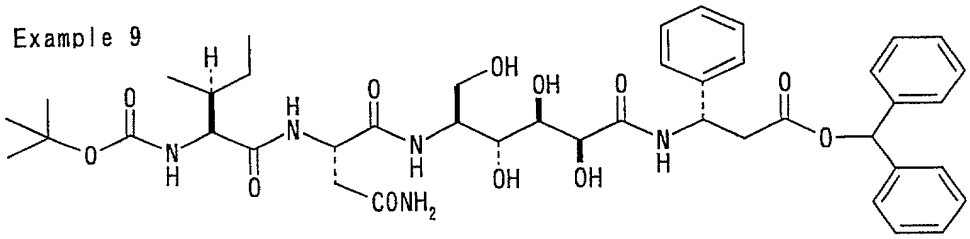

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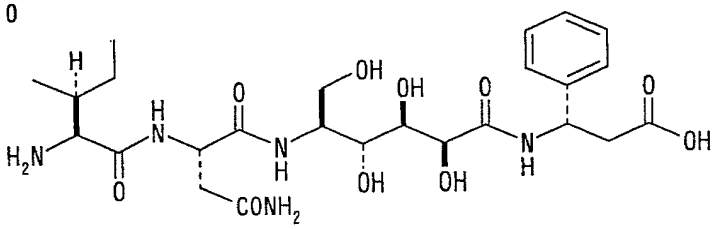

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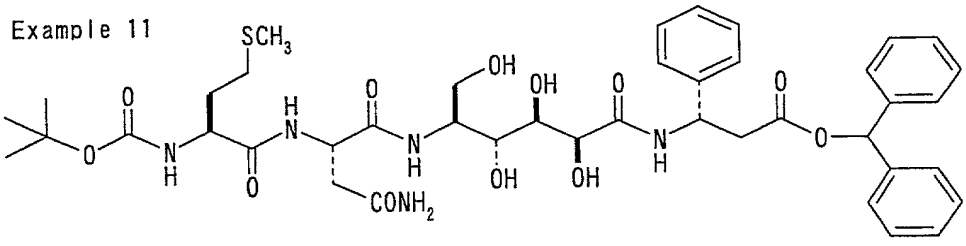

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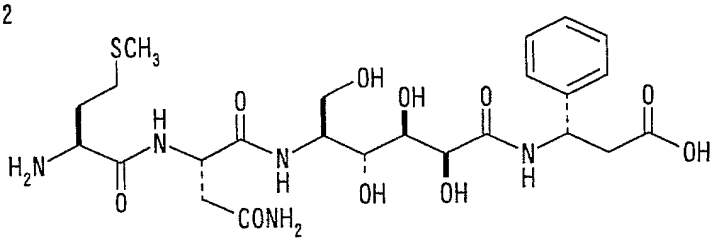

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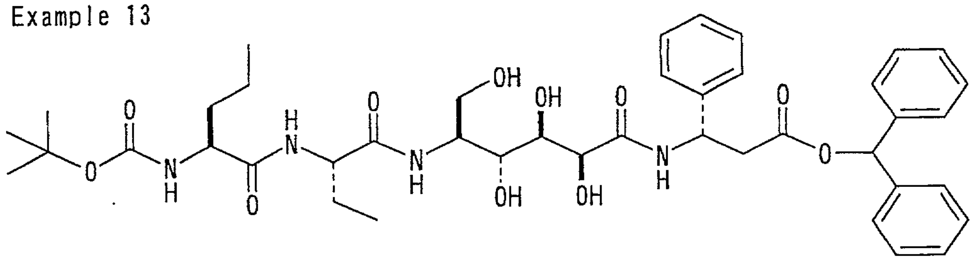

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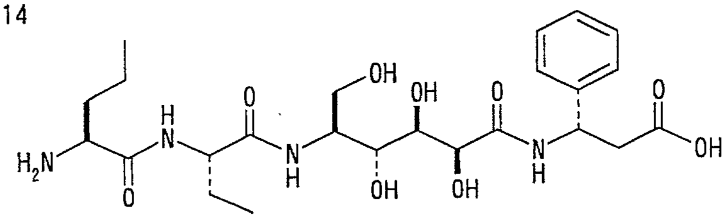

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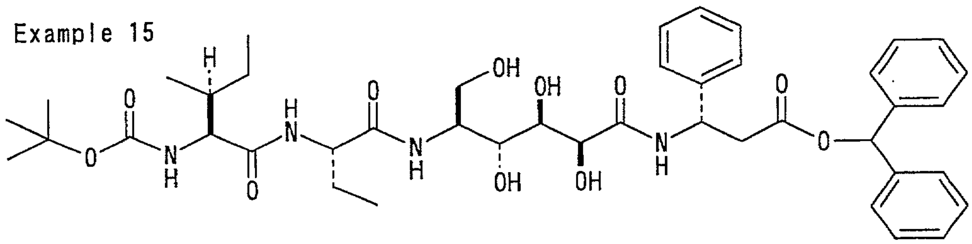

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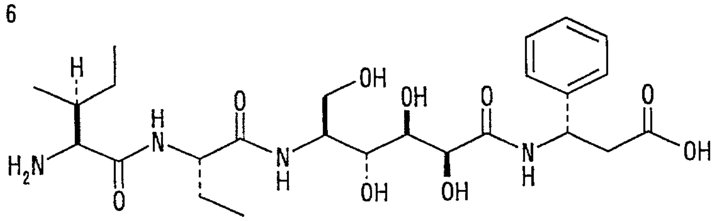

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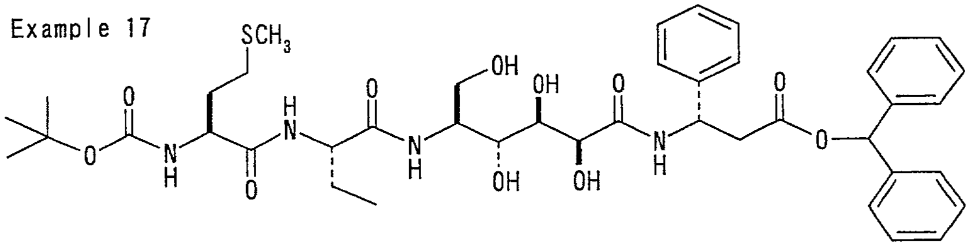

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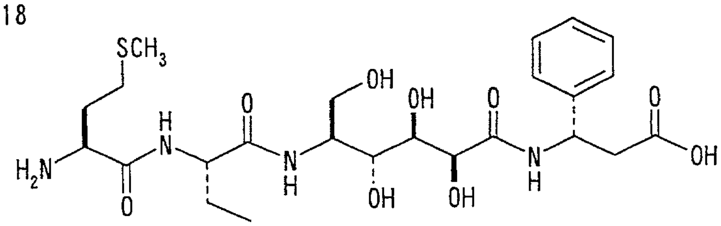

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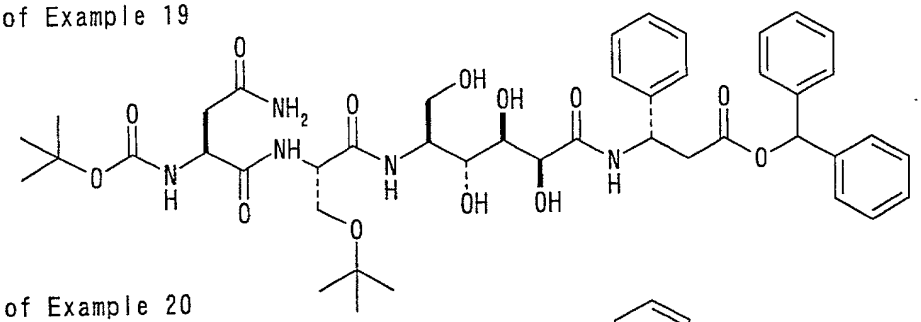

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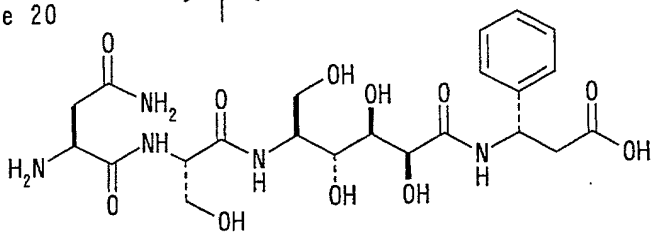

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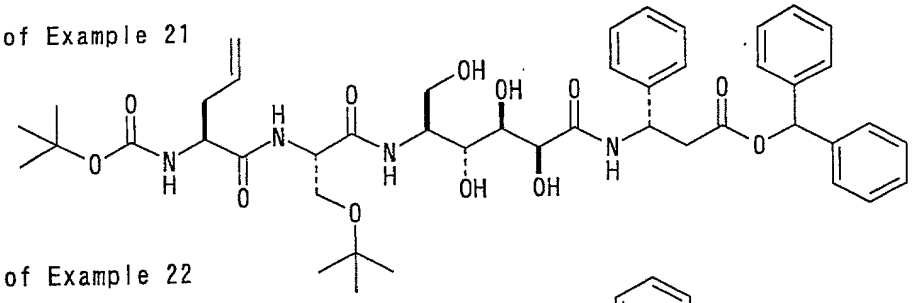

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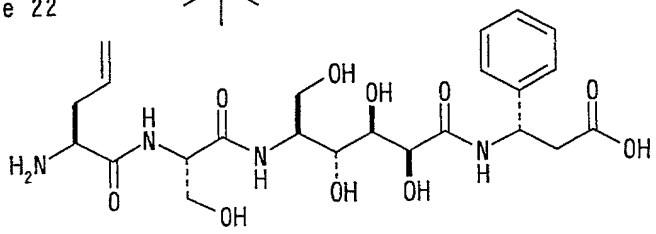

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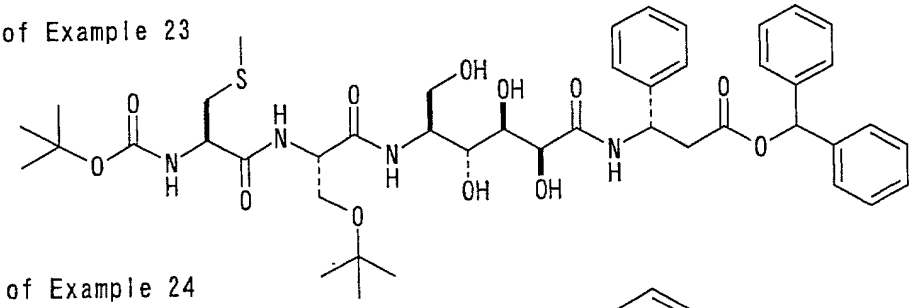

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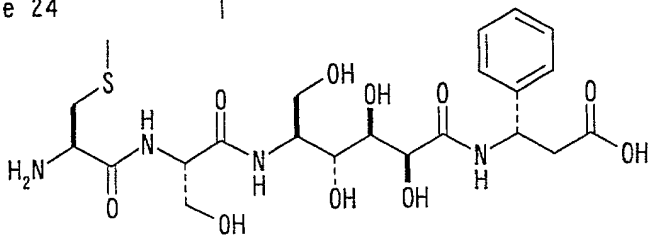

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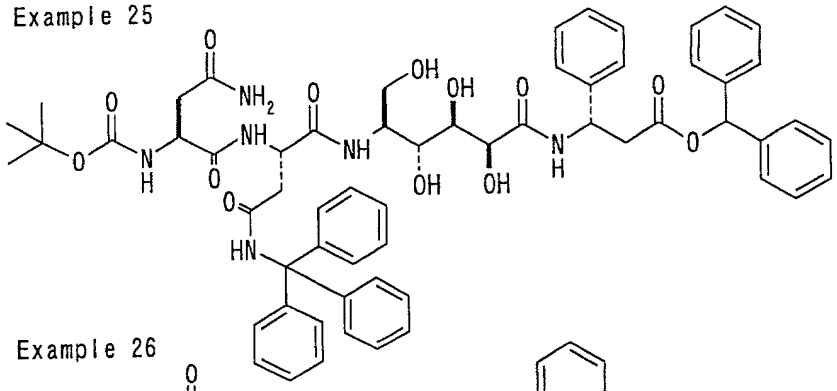

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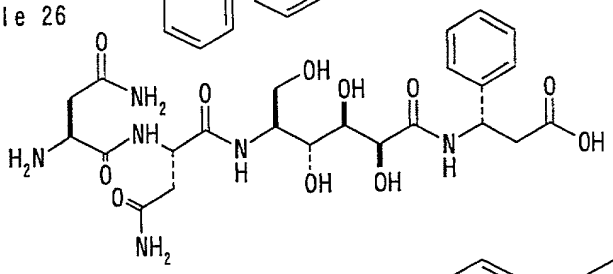

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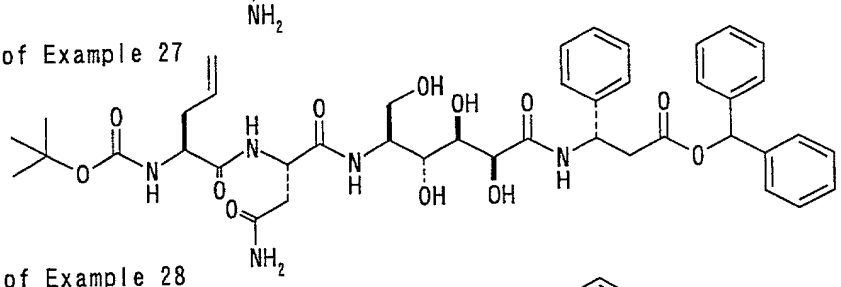

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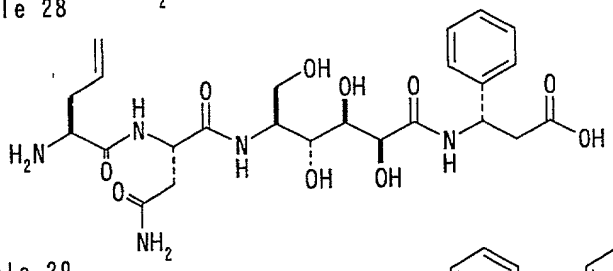

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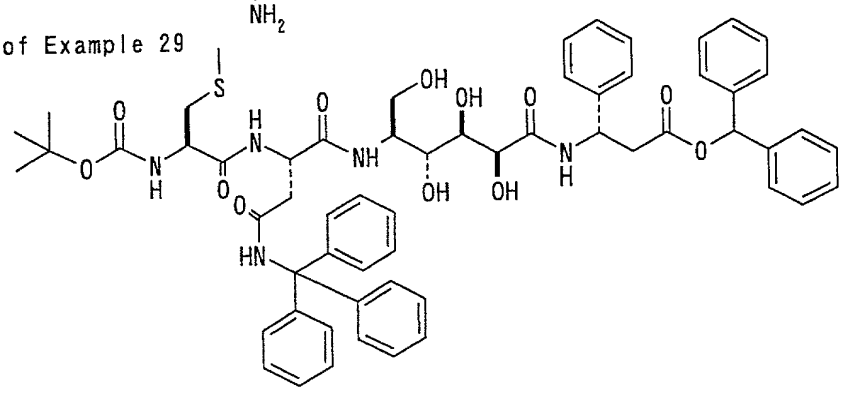

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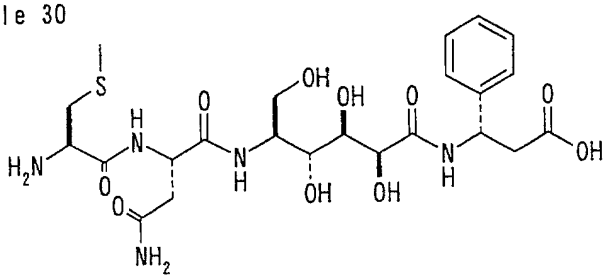

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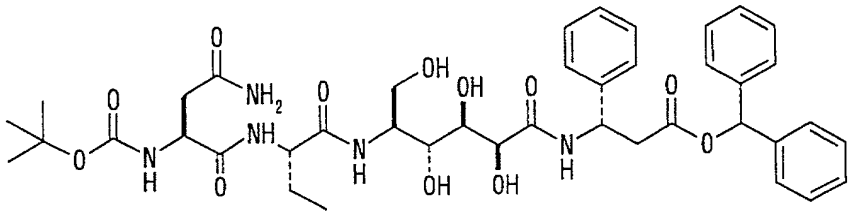
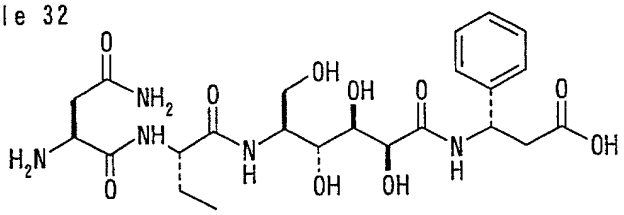


Compound of Example 32



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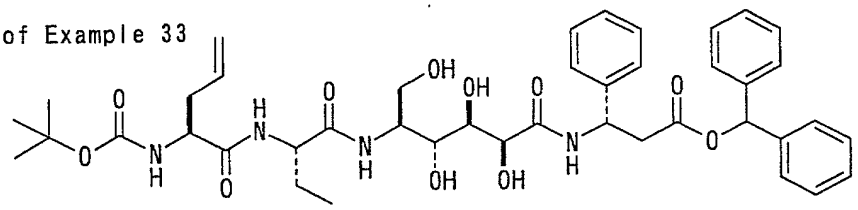
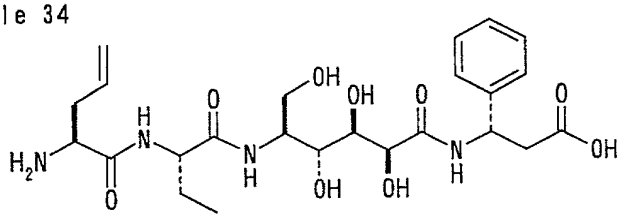


Compound of Example 34



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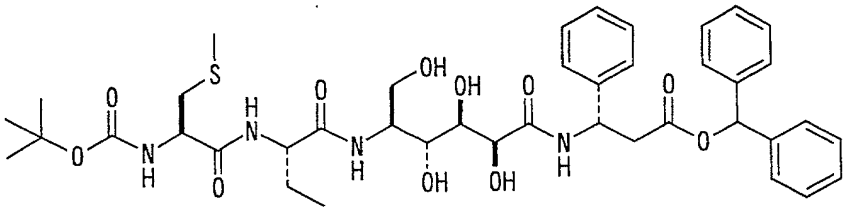

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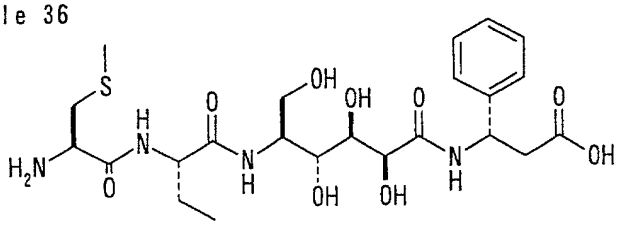

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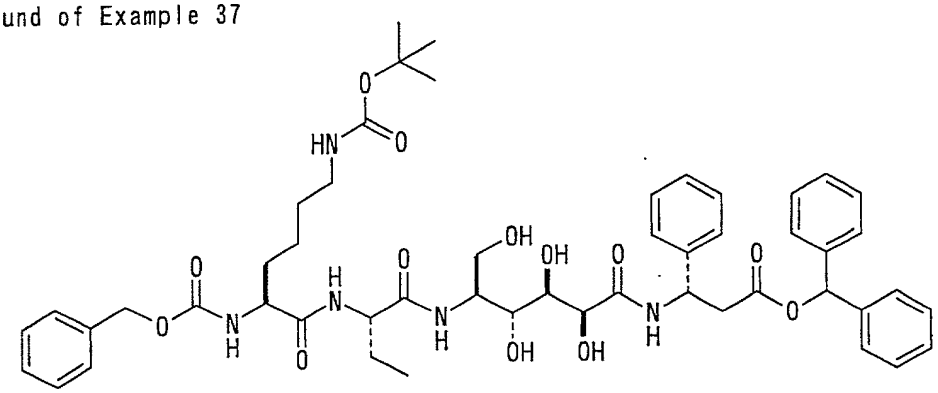

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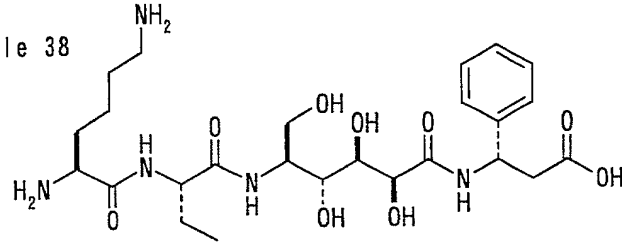
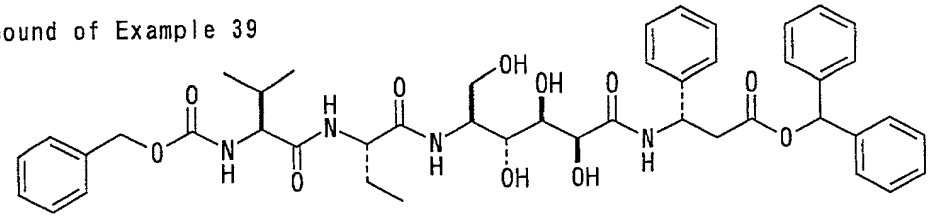
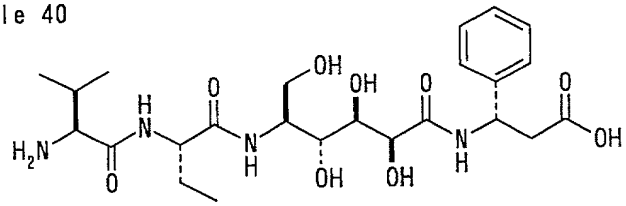


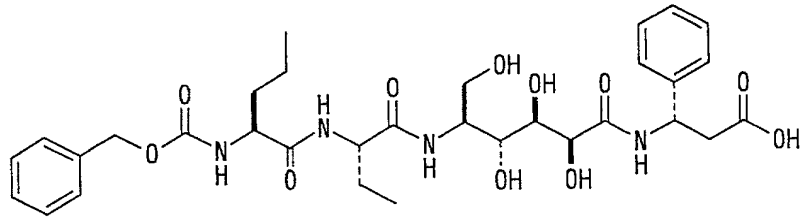
Compound of Example 39



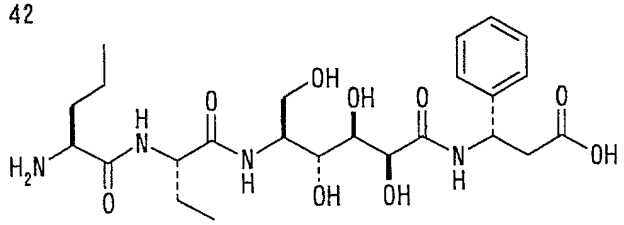
Compound of Example 40



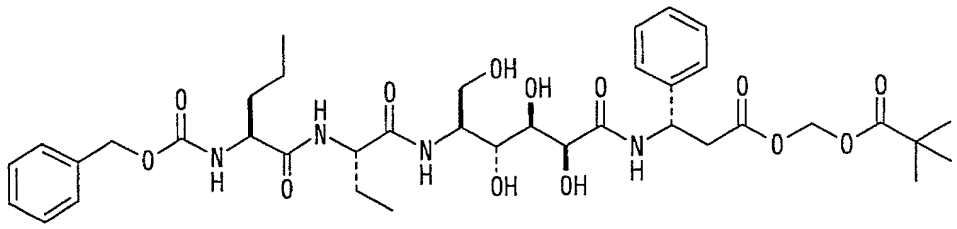
Compound of Example 41



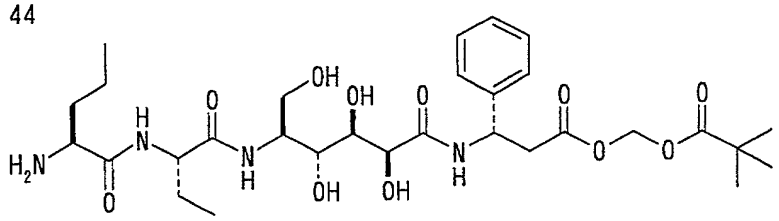
Compound of Example 42



Compound of Example 43



Compound of Example 44



Experiment 1

In vitro antibacterial test: Antibacterial activity against Helicobacter pylori in vitro

Using Helicobacter pylori (NCTC 11637) as the test strain, the antibacterial activity of test compounds was assayed by the agar dilution method as follows. Test compounds were respectively dissolved in dimethyl sulfoxide, and using sterile distilled water, a doubling dilution series was prepared for use as samples. Using 7% horse blood-supplemented Brucella agar as the medium, plates were prepared by mixing 2 mL of each sample with 18 mL of the 7% horse blood-Brucella agar. To prepare an inoculum, Helicobacter pylori was shake-cultured in 2.5% fetal bovine serum-Brucella broth at 37° C for 20 hours using a gas pak jar containing CampyPak™ [BBL Beckton Dickinson Microbiology Systems]. Assay plates were inoculated with 5 μL each of the respective cell suspensions adjusted to about 10^6 CFU/mL with 2.5% fetal bovine serum-Brucella broth and were incubated at 37° C for 4 days in the gas pak jar containing CampyPak™ and water-soaked sanitary cotton. After cultivation, the degree of bacterial growth was grossly evaluated and the minimal concentration at which no growth was observed was recorded as the MIC (minimal inhibitory concentration). The MIC value was <0.006 (μg/mL) for the compound of Example 14 and 0.025 (μg/mL) for the compound of Example 16.

Experiment 2

In vivo antibacterial test:

Mongolian gerbils (MON/Jms/Gbs, male, aged 5 weeks) were deprived of food for 20 hours and $10^{7.58}$ CFU/mouse of Helicobacter pylori TN2GF4 was inoculated into the stomach. Starting 11 days after infection, 50 mg/kg of the test compound suspended in 0.5% methylcellulose/water was administered orally twice daily, in the morning and evening, for 2 consecutive days. On the day following the last dose, the stomach was isolated from the infected gerbils and homogenized and a 10-fold dilution series of the homogenate was inoculated on activated charcoal-modified Skirrow medium. Cultivation was carried out microaerobically at 37° C for 4 days and the eradication rate was determined according to growth of the bacteria.

The results are presented in Table 1. The number of bacteria was expressed in mean ± standard error and the statistical analysis was made in comparison with the control group by the Dunnett method.

Table 1

Sample	Dose (mg/kg)	Clearance rate (%)	Bacteria retrieved (Log CFU/gastric wall)
Control (0.5% methylcellulose)	0	0/4 (0)	6.62±0.06
The compound of Example 14	30	1/4 (25)	2.23±0.48**
The compound of Example 16	30	1/4 (25)	2.25±0.32**

**P<0.01vs control by Dunnett's test

It can be seen from Table 1 that, at the dose level of 30 mg/kg, the compounds of Example 14 and 16 accomplished to reduce the number of recovered Helicobacter pylori. And the compounds of Examples 14 and 16 accomplished 1/4 clearance. It is, therefore, clear that the medicinal composition of the invention is effective in the prevention and treatment of Helicobacter pylori-associated gastritis, gastric ulcer, duodenal ulcer, and cancer of the stomach.

Experiment 3

In vivo anti-Helicobacter pylori effect of the gastric mucosa adhesive preparation

Mongolian gerbils (MON/Jms/Gbs) infected with Helicobacter pylori were orally dosed with the compound of Example 14 containing gastric mucosa adhesive preparation obtained in Formulation Example 2 (the compound of Example 14 AdMMS-1 in Table 2), and a 0.5% methylcellulose suspension containing the compound of Example 14 (the compound of Example 14 suspension in Table 2), respectively at a dose of 3 mg/kg, 10 mg/kg as the compound of Example 14 twice a day for 7 consecutive days. At 16 hours after the final dose, the stomach was excised and the gastric wall was homogenized and serial dilutions were plated on the Helicobacter pylori selective medium. The inoculated medium was incubated for 4 days at 37° C under microaerobic conditions and the number of viable cells was counted. The results are shown in Table 2.

Table 2

Sample	Dose (mg/kg)	Clearance rate (%)	Bacteria retrieved (Log CFU/gastric wall)
Control (0.5% methylcellulose)	0	0/5 (0)	6.15±0.06
The compound of Example 14-ADMMS	3	5/5 (100)	ND**
The compound of Example 14-suspension	10	3/5 (60)	2.18±0.44**

**P<0.01vs control by Dunnett's test; ND: not detected

As shown in Table 2, it is clear that the compound of Example 14 containing gastric mucosa adhesive preparation showed the same level of anti- Helicobacter pylori activity as that of the compound of Example 14 suspension with one third of the dosage of the compound of Example 14 suspension.

Formulation Example 1

For use as a therapeutic agent for Helicobacter pylori infections, the compound or salt of the invention can be administered typically in the following dosage forms.

1. Capsules

(1) Compound obtained in Example 14	100 mg
(2) Lactose	90 mg
(3) Microcrystalline cellulose	70 mg
(4) Magnesium stearate	10 mg
	270 mg per capsule

The whole amounts of (1), (2), and (3) and 1/2 of (4) are blended and granulated. To the granulation is added the remainder of (4) and the whole composition is filled into gelatin capsule shells.

2. Tablets

(1) Compound obtained in Example 14	100 mg
(2) Lactose	35 mg
(3) Corn starch	150 mg
(4) Microcrystalline cellulose	30 mg
(5) Magnesium stearate	5 mg
	320 mg per tablet

The whole amounts of (1), (2) and (3), 2/3 of (4), and 1/2 of (5) are blended and granulated. To the granulation are added the remainders of (4) and (5), and the whole composition is compressed.

Formulation Example 2

Hardened (hydrogenated) caster oil (Lubri wax 101™, Freund Industrial Co. Ltd.) (84 g) was melted at 80 ℃. To this melt, 1 g of compound obtained in Example 14, 10g of acrylic polymer (HIVISWAKO 104™, Wako Pure Chemical Industries, Ltd.) and 5 g of low substituted hydroxypropylcellulose (LH-31™, Shin-Etsu Chemicals) were successively added and the mixture was stirred for dispersion at a constant temperature of 80° C for 2 hours. This molten mixture was dropped onto a 15 cm (di.) aluminum disk rotating at 2400 rpm at a flow rate of 50g/min, whereby spherical fine granules 42 mesh passing through were obtained.

INDUSTRIAL APPLICABILITY

Compound (I) of the invention has specific and high antibacterial activity against Helicobacter bacteria represented by Helicobacter pylori. Therefore, with this Compound (I), the desired anti-Helicobacter pylori efficacy can be achieved at a remarkably reduced dose as compared with the conventional

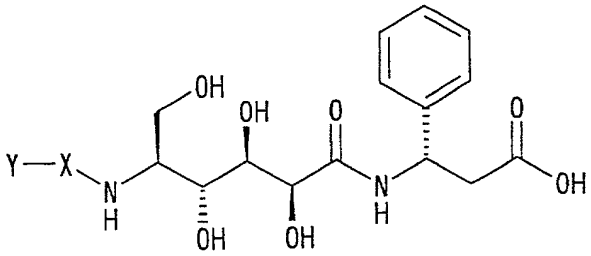
antibacterial agents available for control of Helicobacter bacteria (especially Helicobacter pylori).

Compound (I) is effective in the prevention or treatment of various diseases associated with Helicobacter bacteria, such as duodenal ulcer, gastric ulcer, chronic gastritis, and cancer of the stomach. Moreover, because Helicobacter pylori is a major factor in recurrences of ulcer, Compound (I) is effective in preventing recurrence of ulcers as well.

Furthermore, Compound (I) shows no activity against such gram-positive bacteria as those of the general Staphylococcus and Bacillus, or such gram-negative bacteria as those belonging to the general Escherichia, Pseudomonas, Proteus, Klebsiella, Serratia, Salmonella, Citrobacter, Alcaligenes, etc. Therefore, Compound (I) is selectively effective in the prevention or treatment of diseases associated with Helicobacter bacteria, with minimal effects on other bacteria and fungi, and, therefore, can be used as a safe drug.

CLAIMS

1. A compound of the formula:



(I)

[wherein X is L-serine residue, L-asparagine residue or (S)-2-aminobutyric acid residue and Y is α-L-amino acid residue] or its salt.

2. A compound as claimed in claim 1, wherein X is (S)-2-aminobutyric acid residue.

3. A compound as claimed in claim 1, wherein Y is norvaline residue, isoleucine residue or methionine residue.

4. A compound as claimed in claim 1, which is (S)-3-[(2S,3R,4R,5S)-5-(L-norvalyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid or its salt.

5. A compound as claimed in claim 1, which is (S)-3-[(2S,3R,4R,5S)-5-(L-isoleucyl-(S)-2-aminobutyryl)amino-2,3,4,6-tetrahydroxyhexanoyl]amino-3-phenylpropionic acid or its salt.

6. A pro-drug of the compound claimed in claim 1.

7. A pharmaceutical composition which contains the compound claimed in claim 1 or its pro-drug.

8. A pharmaceutical composition as claimed in claim 7, which is an anti-Helicobacter pylori agent.

9. A pharmaceutical composition as claimed in claim 8, which is a preventing and treating agent of Helicobacter pylori infectious disease.

10. A pharmaceutical composition as claimed in claim 9, wherein Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

11. A pharmaceutical composition as claimed in claim 7, which

is a gastric mucosa adhesive pharmaceutical composition.

12. A pharmaceutical composition as claimed in claim 11, wherein a gastric mucosa adhesive pharmaceutical composition contains (a) a compound as claimed in claim 1, (b) a lipid and/or a polyglycerol fatty acid ester and (c) a viscogenic agent capable of being viscous with water.

13. A pharmaceutical composition as claimed in claim 12, wherein (c) the viscogenic agent is an acrylic polymer.

14. A pharmaceutical composition as claimed in claim 12, which further contains (d) a material which swells the viscogenic agent.

15. A pharmaceutical composition as claimed in claim 14, (d) the material which swells the viscogenic agent is curdlan and/or a low-substituted hydroxypropylcellulose.

16. A pharmaceutical composition which contains both of a compound as claimed in claim 1 or its pro-drug and the other antibacterial agent and/or an antiulcerative agent.

17. A method for treating or preventing a mammal suffering from a Helicobacter pylori infectious disease, which comprises administering an effective amount of a compound according to claim 1 or its pro-drug optionally together with a pharmaceutically acceptable carrier, diluent or excipient, to a patient suffering from the disease.

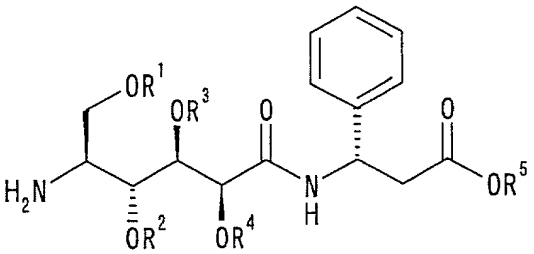
18. A method as claimed in claim 17, wherein Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

19. Use of the compound according to claim 1 or its pro-drug for manufacturing of a pharmaceutical composition for a Helicobacter pylori infectious disease.

20. Use as claimed in claim 19, wherein the composition is for treating or preventing a Helicobacter pylori infectious disease.

21. Use as claimed in claim 20, wherein the Helicobacter pylori infectious disease is gastric or duodenal ulcer, gastritis, gastric cancer or gastric MALT lymphoma.

22. A method for producing a compound claimed in claim 1, which comprises reacting a compound of the formula:



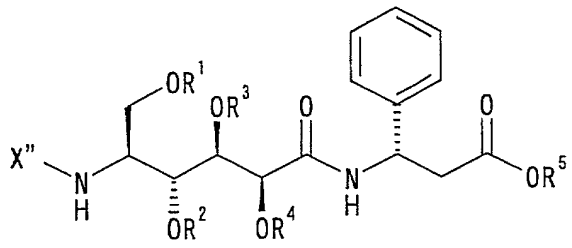
(II)

[wherein R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom], its salt or its reactive derivative at the amino group with a compound of the formula:

$$Y'—X'—OH \qquad (III)$$

[wherein X' is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, and Y' is α-L-amino acid residue which may be protected], its salt or its reactive derivative at the carboxyl group, if necessary, followed by removing the protecting group.

23. A method for producing a compound claimed in claim 1, which comprises reacting a compound of the formula:



(IV)

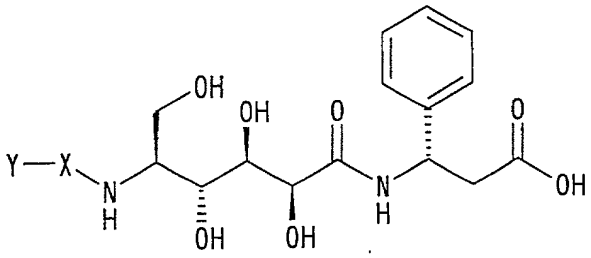
[wherein X" is L-serine residue which may be protected, L-asparagine residue which may be protected or (S)-2-aminobutyric acid residue, R^1, R^2, R^3 and R^4 are independently a protecting group for hydroxy group or a hydrogen atom, and R^5 is a protecting group for carboxyl group or a hydrogen atom], its salt or its reactive derivative at the amino group with a compound of the formula:

$$Y'—OH \qquad (V)$$

[wherein Y' is α-L-amino acid residue which may be protected], its salt or its reactive derivative at the carboxyl group, if necessary, followed by removing the protecting group.

ABSTRACT

A compound of the formula:



(I)

wherein X is L-serine residue, L-asparagine residue or (S)-2-aminobutyric acid residue and Y is α-L-amino acid residue, its salt or its pro-drug has remarkable antibacterial activity against Helicobacter bacteria.

Rev. 1/1/99 Effective October 20, 1997

DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATION

( ) Original ( ) Supplemental ( ) Substitute (X) PCT ( ) Design

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Title: POLYOL COMPOUNDS, THEIR PRODUCTION AND USE

of which is described and claimed in:
( ) the attached specification, or
( ) the specification in the application Serial No. _____ filed _____;
and with amendments through _____ (if applicable), or
(X) the specification in International Application No. PCT/ JP00/00023 , filed January 6, 2000 , and as amended on _____ (if applicable).

I hereby state that I have reviewed and understand the content of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge my duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, ' 1.56.

I hereby claim priority benefits under Title 35, United States Code, ' 119 (and ' 172 if this application is for a Design) of any application(s) for patent or inventor's certificate listed below and have also identified below any application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NO.	DATE OF FILING	PRIORITY CLAIMED
Japan	001898/1999	January 7, 1999	YES

I hereby claim the benefit under Title 35, United States Code ' 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code ' 112, I acknowledge the duty to disclose information material to patentability as defined in Title 37, Code of Federal Regulations, ' 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

APPLICATION SERIAL NO.	U.S. FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

And I hereby appoint Michael R. Davis, Reg. No. 25,134; Matthew M. Jacob, Reg. No. 25,154; Jeffrey Nolton, Reg. No. 25,408; Warren M. Cheek, Jr., Reg. No. 33,367; Nils E. Pedersen, Reg. No. 33,145; and, Charles R. Watts, Reg. No. 33,142, who together constitute the firm of WENDEROTH, LIND & PONACK, L.L.P., jointly and severally, attorneys to prosecute this application and to transact all business in the U.S. Patent and Trademark Office connected therewith.

I hereby authorize the U.S. attorneys named herein to accept and follow instructions from AOYAMA & PARTNERS as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and myself. In the event of a change in the persons from whom instructions may be taken, the U.S. attorneys named herein will be so notified by me.

ATTACHMENT B

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I further declare that all statements made herein of my own knowledge are true, and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Keiji KAMIYAMA

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Ken-ichiro MIYAGAWA

6th Inventor Yohko Akiyama Date May 25 2001
Yohko AKIYAMA

7th Inventor _____ Date _____

The above application may be more particularly identified as follows:

U.S. Application Serial No. _____ Filing Date _____

Applicant Reference Number _____ Atty Docket No. _____

Title of Invention _____

_____